A SURVEY OF MICROBIAL INOCULANTS FOR BIOREMEDIATION AND IDENTIFICATION OF INFORMATION REQUIREMENTS SUITABLE FOR THE FEASIBILITY EVALUATION AND VALIDATION OF BIOREMEDIATION

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Report prepared for:

Hazardous Contaminants Branch
Ontario Ministry of the Environment

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EXECUTIVE SUMMARY

Bioremediation is a managed or spontaneous biological processes (usually microbiological) that can be used to detoxify contaminated soils and/or waters. Bioremediation is increasingly finding favour with regulators and industry as it is a cost effective remedial technology. However, users of bioremedial technology generally lack proper understanding of this technology and thus lack the ability to properly assess the feasibility of applying this technology. In addition, users of bioremedial technology generally lack the ability to assess if (and which) allochthonous microorganisms should be added in a bioremedial strategy. Beak Consultants Limited (BEAK) was contracted by the Ontario Ministry of the Environment to develop a reference source which summarizes the microorganisms currently used or expected to be used for bioremediation, and to develop a criteria document for evaluating bioremediation (*a priori* and *post priori*).

An exhaustive survey of the Canadian, U.S. and international private sectors and research institutions was undertaken to determine current uses of bioremediation. Two hundred and one (201) individuals representing 189 commercial organizations were invited to respond to a questionnaire. A total of 53 (28%) commercial organizations responded to the survey. Of the responding organizations, 37 *practice* (apply) bioremediation and 20 use or have used allochthonous microorganisms in bioremediation projects. Only 4 responding organizations were solely involved in the production and supply of microbial inoculants. Several organizations do not manufacture their own inocula but obtain it from another firm under a private label. The relationship of these companies is confidential.

Thirty (30) Canadian organizations, identified as potential bioremediation users, were invited to respond to a questionnaire. Of the ten (10) responding organizations, 9 are involved in bioremediation, 5 use (add) allochthonous microorganisms and 3 supply allochthonous microorganisms. None of the responding Canadian organizations are solely involved in the manufacture and supply of microorganisms.

Of the approximately 75 commercial inoculants identified, 31% are for use in biodegradation of aliphatics, namely petroleum hydrocarbons. Less than 2% of inoculants were suggested for use in biodegradation of halogenated polyaromatics such as polychlorinated biphenyls (PCBs). Similarly, only 6% of inoculants are suggested for biodegradation of polycyclics (often pesticides), while 9% of inoculants were suggested for use in biodegradation of halogenated

aliphatics such as trichloroethene and other solvents. No inoculants were suggested for use in the bioremediation of metal contamination.

Microorganisms of the genus *Pseudomonas* were the most commonly used microorganisms in the microbial inoculants reviewed, likely owing to their ecological fitness and competitive abilities. *Bacillus*, *Mycobacterium* and *Micrococcus* were also commonly cited as contents of microbial inoculants. Other microorganisms used in the inoculants reviewed include: *Phanaerochaetes*, *Alcaligenes*, *Nocardia*, *Rhodococcus*, *Acinetobacter*, *Thiobacillus*, *Psychrobacter*, *Arthrobacter*, *Actinomycetes*, *Flavobacterium*, *Methylosinus*, *Saccharomyces*, *Nitrobacter*, *Nitrosomonas*, *Aspergillus*, *Myxobacter* and *Corynebacter*.

With respect to applications, 72% of the inoculants reviewed are recommended for use in on-site applications (*e.g.*, bioreactors, composting) while 79% of the inoculants reviewed are recommended for *in situ* bioremediation (soil and groundwater cleanup, landfarming).

Three active areas of microbial inoculant research were identified. These include:

- isolation of additional naturally occurring microorganisms capable of biodegrading targeted pollutants;
- improvement of existing and novel microorganisms to enhance their competitive and catabolic ability in natural environments. Enhancement may include improving the characteristics of the microorganisms through genetic engineering and selection or by identifying and controlling the factors that affect their survival and activity; and
- development of non-aqueous microbial formulations and carriers which increase the stability and success of introduced microorganisms.

A number of researchers contacted through the inoculant survey raised concerns over the efficacy of microbial inoculants. The ability of added microorganisms to effectively outcompete indigenous microorganisms seems to be of great debate. Public and environmental health concerns were also raised regarding the addition of allochthonous microorganisms. The concerns and considerations in the use of commercial inoculants are discussed in detail within this document.

One hundred and thirty-six (136) individuals representing 116 research institutions were contacted and asked to identify information and criteria which they feel are important in evaluating bioremediation (both *a priori* and *post priori*). From the responses of these researchers and the available scientific literature, BEAK was able to cull the information which should be evaluated (termed herein as "information requirements") prior to choosing bioremediation as a treatment strategy or prior to designing a bioremediation monitoring program. From these factors, BEAK has developed a suggested step-wise process for evaluation of bioremediation accompanied by a comprehensive flowchart which could be used to quickly process information during evaluation or monitoring of bioremediation.

It is important to note that not all the suggested information requirements are needed to evaluate bioremediation. The required information and criteria will be case specific (*i.e.*, site specific). In addition, many information requirements are highly interrelated and thus they should not be considered in isolation. It may be possible to infer, or fill in, missing information, or to "logically link indirect evidence" to come to a conclusion on the feasibility or success of bioremediation. This will be particularly true in cases where collection of all suggested information is not feasible or when it is not possible to obtain or estimate realistic values for the information requirements identified.

The suggested information requirements have been selected to encompass both on-site and *in situ* applications of bioremediation. However, in several cases an information requirement may be exclusive, or more applicable, to either on-site or *in situ* bioremediation. Similarly, specific information requirements will be exclusive to either evaluating or monitoring bioremediation. Information requirements which are exclusive are appropriately noted.

The suggested information requirements are divided into three main categories, which are;

- contaminant(s);
- site physicochemical parameters; and
- microbial characterization.

Characterization of the contaminants involves:

- identification of the chemicals to be bioremediated;

- identification of their chemical attributes (*e.g.*, chemical structure, solubility, volatility, sorption and chemical reaction);
- assessing their biodegradability (*e.g.*, biodegradation rates and kinetics, toxicity, and concentration thresholds); and
- determination of the distribution and mass.

Characterization of the physicochemical attributes of the contaminated environment consist of:

- characterizing the geochemistry of the contaminated environment (*e.g.*, pH, Eh/redox conditions, temperature, osmotic pressure, moisture content, and nutrients/electron acceptors); and
- characterization of the hydrogeological/geological conditions (*e.g.*, delineation of the saturated and unsaturated zones, estimation of the depth to the watertable, measurement of the hydraulic parameters (K, gradient, porosity and permeability) and measurement of the cation exchange capacity, the particle size distribution and the natural organic carbon content).

Characterization of the microbiology of the contaminated environment involves assessment of:

- the initial biomass size;
- the community composition and its distribution; and
- the initial microbial activity and the potential to stimulate the desired metabolic activity.

Of note with respect to microbiological characterization is the use of newer monitoring/assessment techniques, such as phospholipid fatty acid (PLFA) analysis and genetic probes to obtain representative microbial measurements while circumventing the limitations (*e.g.*, culturing) imposed by classical culturing techniques.

Seven general steps for evaluating the feasibility of bioremediation are identified and discussed. The use of these steps, accompanied by a suggested flowchart, should assist in processing information during evaluation or monitoring of bioremediation. The seven steps are:

1) Identification and characterization of the contaminant(s).

2) Assessment of contaminant biodegradability through scientific literature and laboratory treatability studies. These tests yield data that addresses the potential of a proposed bioremediation strategy to meet performance goals. Laboratory screening should provide "yes/no" determinations of the validity of a suggested biotreatment process and should quickly screen out inappropriate strategies prior to their evaluation by costly bench- and/or pilot-scale studies. Laboratory treatability studies should also identify parameters (*i.e.*, reaction mechanisms, transport pathways) that will require specific attention during bench- and/or pilot-scale testing and should direct the focus of these subsequent studies.

3) Characterization of the contaminated environment including geochemical, hydrogeological and microbial characterization.

4) Bench-scale treatability studies to assess case-specific biodegradation using the proposed bioremedial strategy. Bench-scale treatability systems should be representative systems designed to verify that the technology selected can meet expected cleanup goals. These systems should generate cost and design data and should allow design of an appropriate pilot-scale (*i.e.*, field) program based on sound science and engineering.

5) Assessment of potential biostimulation and feasibility of adjusting environmental conditions.

6) Pilot-scale (field) testing of bioremediation scenarios to determine time required for cleanup, level of attainable cleanup, and cost of cleanup. Pilot-scale testing should consider field-scale limitations imposed by geochemical and/or hydrogeological constraints (*i.e.*, soil, chemical, and biological heterogeneity, permeability) and mass transfer and fluid transport limitations. The scope of the pilot test should be large enough that realistic complications will be encountered. Ultimately, pilot-scale testing, and the entire stepwise process outlined, should yield the most reliable, time and cost effective strategy for bioremediation.

7) Monitoring to validate bioremediation.

Various laboratory (microcosm) and field (*in situ* testers) methods are discussed with particular attention on how to improve the interpretation of laboratory data and how to apply such data to field applications.

In summary, this report presents considerations for evaluating bioremediation and microbial inoculants from primarily an efficacy standpoint although several environmental and public health issues have also been addressed regarding the use of microbial inoculants. The considerations discussed herein are based on the latest available data. As with any emerging technology, new field and laboratory research will generate data that may result in the addition, adaptation or deletion of information requirements. Only through familiarity with existing information requirements and developments in laboratory and field research, can the minimum information required to evaluate bioremediation, both *a priori* and *post priori*, be ascertained.

1.0 INTRODUCTION

Increased use of bioremediation in the cleanup of environmental contamination has led to concern over the efficacy of biologically-mediated treatments methods. The establishment and continual expansion of a biological treatment industry, has increased the importance of identifying information requirements and criteria which can be used to evaluate bioremediation proposals and to validate bioremediation claims.

Bioremediation refers to a managed or spontaneous process in which biological, especially microbiological, catalysis acts on chemical compounds, thereby detoxifying or eliminating environmental contamination. This may involve biodegradation, which is the complete conversion of the contaminant to inorganic by-products such as carbon dioxide and water (*i.e.*, mineralization) or may involve conversion of the contaminants to other compounds (*i.e.*, biotransformation). Bioremediation synonyms include, bioreclamation, biostimulation and biotreatment. Each term is defined in the Glossary of Terms in Appendix A.

Current bioremediation applications can be divided into three separate processes, designated in this report as off-site, on-site and *in situ*. Off-site biological treatment consists of methods practised at waste treatment facilities and sewage treatment plants, for which regulatory guidelines and controls have been firmly established. Off-site biological treatment will not be addressed in this report.

On-site biological treatment involves removal of contaminated materials, generally by excavation of soils or pumping of groundwater, followed by their treatment at the contaminated site. On-site applications may include composting or treatment in any engineered system such as biofilm or bioslurry reactors. The use of commercial inoculants is often associated with on-site treatments as desirable allochthonous microorganisms can be added to engineered systems or compost piles to enhance biodegradation.

In situ bioremediation is defined as the enhancement of biological (*e.g.*, degradative) activity in soils and/or groundwater *in place*. Many organizations incorrectly define the bioremediation process as *in situ* when it is actually an on-site treatment. *In situ* bioremediation may involve manipulation of geochemical and/or environmental conditions, addition of selected, adapted or engineered microorganisms and/or pumping of groundwater for the purpose of hydrogeological

control or nutrient addition but does not involve contaminant treatment in engineered systems.

In recent years, the use of on-site and *in situ* bioremediation has increased but questions on the efficacy of the processes still exist. Lacking has been scientifically sound information on which to base regulatory decisions for the use of bioremediation. Specifically lacking are guidelines to determine if bioremediation, as opposed to other physicochemical process(es), has been responsible for contaminant removal, degradation and/or detoxification. Guidelines are also lacking for evaluating the efficacy of inoculants to enhance bioremediation.

1.1 Objectives of the Study

The objective of this report is two-fold; one, to develop a reference source which summarizes microorganisms currently used or expected to be used for bioremediation, and second, to develop a criteria document for evaluating bioremediation (*a priori* and *post priori*). The document provides the framework within which bioremediation proposals and claims can be evaluated. The framework has been divided into three major divisions: inoculants (Chapter 2.0), information requirements (Chapter 3.0), and evaluation of bioremediation (Chapter 4.0).

Chapter 2.0 examines commercial inoculants currently available and provides a summary of their contents, and their current suggested uses. Future directions in the production, type and use of inoculants is addressed. An extensive discussion on the factors that should be considered in the use of inoculants is also provided.

Chapter 3.0 presents the suggested information requirements and criteria, and information on processes which could be used for evaluation and monitoring of bioremediation. The rationale for each suggested information requirement is discussed and fully referenced. The suggested information requirements have been selected to encompass both on-site and *in situ* applications of bioremediation. However, in several cases an information requirement may be exclusive, or more applicable, to either on-site or *in situ* bioremediation. Similarly, specific information requirements will be exclusive to either evaluating or monitoring bioremediation. Information requirements which are exclusive are appropriately noted.

Chapter 4.0 presents a suggested step-wise process for evaluation of bioremediation and is accompanied by a comprehensive flowchart which could be used to quickly process information

during evaluation or monitoring of bioremediation. All information requirements and processes in the flowchart are cross referenced to the specific sections within the document.

Beak Consultants Limited (BEAK) believes that this document should be useful in determining when bioremediation is feasible, and when bioremediation should not be applied.

1.2 Study Approach

1.2.1 Survey of Microbial Inoculants for Bioremediation

An exhaustive survey of the Canadian, U.S. and international private sectors and research institutions was undertaken to determine current uses of bioremediation. Identification of enterprises and agencies was made through contacts of Beak Consultants Limited (BEAK), a review of the current bioremediation journals, books, government agency reports, conference proceedings and company promotional material. Enterprises were also identified through international symposium lists and referrals from respondents. Each reference has been properly cited including author, title and source.

Two hundred and one (201) individuals representing 189 organizations were invited to respond to a questionnaire (Appendix B). In several cases, more than one office for an organization was contacted. Contacts were asked to indicate whether the organizations they represented practised bioremediation and whether they used allochthonous microorganisms in their bioremediation practices. Organizations making allochthonous additions were asked to identify (genus, species) the microorganisms which they used and their source of inoculum. Contacts were also asked to list the chemicals that were or could be degraded by the microorganisms as well as the applications for which the microorganisms were or could be used (*i.e.*, On-site versus *in situ* treatments).

The extant scientific literature was also surveyed to identify microbial inoculants that could be used in the future. In particular, attention was given to microbial species that are or could be genetically engineered to possess useful catabolic activities or enhanced survival capabilities.

1.2.2 Development of Information Requirements and Criteria for Bioremediation.

One hundred and thirty-six (136) individuals representing 116 research institutions were contacted and asked to identify information and criteria which they feel are important in evaluating and/or monitoring bioremediation. In several cases, more than one individual was contacted at a research institution. Individuals were also asked to suggest appropriate endpoints for the information requirements and were invited to submit any literature, both published and/or unpublished (*e.g.*, manuscripts), which they felt would support their decisions or fill our knowledge base.

As a result of the survey, BEAK was able to obtain references and copies of obscure (*i.e.*, trade journal) articles, internal progress reports and final reports, promotional materials, unpublished manuscripts (in preparation or submitted), paper presentations, conference proceedings, theses, case histories and personal communications.

Based on the information obtained and theoretical considerations, scientifically sound information requirements and criteria were developed. These suggested information requirements and criteria could be used to evaluate the feasibility of applying bioremediation as well as to monitor and validate bioremediation. Various protocols for collecting the appropriate data were also identified. A generalized flowchart was developed to assist in the assessment of the suggested information requirements.

2.0 MICROBIAL INOCULANTS FOR BIOREMEDIATION

2.1 Organizations Involved in Bioremediation

Of the 189 commercial organizations contacted, 53 (28%) responded to the questionnaire. Table 2.1 lists responding organizations and indicates the involvement of these organizations in bioremediation. Table 2.2 summarizes the bioremediation involvement of the organizations listed in Table 2.1 and includes a breakdown of survey results in terms of Canadian and International (non-North American) organizations. Appendix C provides a complete list of organizations and individuals contacted for the microbial inoculant survey.

Table 2.2 shows that 41 of the 53 (77%) responding organizations are *involved* in bioremediation. The involvement of these organizations can be categorized as follows:

- project management of bioremediation;
- manufacture and sale of allochthonous microorganisms; and
- treatability and feasibility studies.

Several organizations are involved in more than one aspect, for example, some organizations manufacture allochthonous microorganisms and manage bioremediation projects. In addition to the above categories, several organizations were identified as manufacturers of specific nutrient amendments and enhancing elements to aid bioremediation processes.

Of the 41 responding organizations involved in bioremediation, 37 (90%) *practice* (apply) bioremediation (Table 2.2). Of these 37 organizations, 20 (54%) use or have used allochthonous microorganisms in bioremediation projects (Table 2.2). This includes organizations who have isolated a microorganism or consortium from a site, cultured (with or without enhancing) the desired microorganism(s), and inoculated the microorganism(s) back at the site (or any other site). While the microorganisms are not novel to the site, they are considered as allochthonous in this report (see section 2.2).

The balance of organizations (46%) practising bioremediation prefer to use indigenous microorganisms in the remediation of contaminants. Many respondents raised questions about the efficacy of allochthonous microorganisms. A review of respondents comments on the benefits and disadvantages of commercial inoculants is provided in Section 2.4.

A total of 20 organizations supply allochthonous microorganisms for bioremediation, however, only 4 (20%) of these organizations are involved solely in the production and supply of these microorganisms (Table 2.2). The remaining 16 (80%) organizations perform treatability studies and apply bioremediation using their own microbial inoculants (Table 2.2). Several of these companies do not manufacture their own inocula but obtain it from another firm under a private label. The relationship of these companies is confidential.

In terms of Canadian involvement in bioremediation, 33% of commercial organizations contacted responded to the questionnaire. Of the ten (10) responding organizations, 90% are involved in bioremediation, 50% add allochthonous microorganisms and 30% supply allochthonous microorganisms. None of the responding organizations are solely involved in the manufacture and supply of microorganisms (Table 2.2).

2.2 Survey of Commercial Inoculants

Table 2.3 provides details on the types of allochthonous microorganisms which are "manufactured" and supplied for bioremediation. In cases where the information was not proprietary, the source of microorganisms was identified. Several organizations were hesitant to divulge the taxonomic contents of their formulations and as such provided only trade names or identified the contents as a consortia. However, more taxonomic information than we expected was provided and as such, Table 2.3 provides a reasonable overview as to the microbial contents of inoculants currently available.

Figure 2.1 shows the frequency of citation of microorganisms in the inoculants in Table 2.3. *Pseudomonas* is the most commonly cited genera at 33%, followed by *Bacillus* at 13%, *Mycobacterium* and *Micrococcus* at 6% and *Phanaerochaetes*, *Alcaligenes*, *Nocardia*, *Rhodococcus* and *Acinetobacter* each at 4% (Table 2.3). The remaining 22% of citations consisted of individual citations of *Thiobacillus*, *Psychrobacter*, *Arthrobacter*, *Actinomycetes*, *Flavobacterium*, *Methylosinus*, *Saccharomyces*, *Nitrobacter*, *Nitrosomonas*, *Aspergillus*, *Myxobacter* and *Corynebacter* (Table 2.3).

Table 2.4 provides ecological profiles for each of the microbial genera identified in Table 2.3. A description of the genus, its typical habitat, enzymatic capabilities, limitations and potential substrates are given. Concerns over the use of the microorganisms, particularly with respect to environmental and public health are also provided.

A common practice among organizations involved in bioremediation is to isolate microorganisms which degrade certain types of contamination. For example, an organization may isolate a particular hydrocarbon degrader or consortia from a site contaminated with hydrocarbons. These microorganisms are cultured in the laboratory and their abilities are often selectively enhanced. The microorganisms are then introduced back at the site in numbers far exceeding their natural carrying capacity. In this manner, the numbers and the biodegradative abilities of the microbial population originally present at the site have been improved. In many cases, the cultured microorganisms are used as inocula for subsequent bioremediation activities. For the purpose of this report, microorganisms isolated from a contaminated environment, cultured and used as an inoculant are considered allochthonous microorganisms.

2.3 Application of Commercial Inoculants

A significant number of recognized pollutant compounds can be biodegraded or biotransformed by microorganisms. Table 2.5 lists examples of published microbial biotransformations derived through laboratory degradation studies. Unfortunately, few of these chemicals have been biotransformed in field environments (microcosm and/or small-scale field trials) and a paucity of data exists on the survival, activity and competitive ability of the listed microorganisms. The value of this list is that it provides an idea of what microorganisms we may begin to see in commercial inoculants and in bioremediation ventures.

Figure 2.2 shows the frequency of citation of chemical classes which can be biodegraded by the inoculants listed in Table 2.3. Biodegradation of these chemical classes is discussed in section 3.1.2.1. Of the approximately 75 commercial inoculants identified, 31% are for use in biodegradation of aliphatics, namely petroleum hydrocarbons. Less than 2% of inoculants were suggested for use in biodegradation of halogenated polyaromatics such as polychlorinated biphenyls (PCBs). Similarly, only 6% of inoculants are suggested for biodegradation of polycyclics (often pesticides), while 9% of inoculants were suggested for use in biodegradation of halogenated aliphatics such as trichloroethene and other solvents. No inoculants were suggested for use in the bioremediation of metal contamination.

With respect to applications, 72% of the inoculants listed in Table 2.3 are recommended for use in on-site applications (*e.g.*, bioreactors, composting) while 79% of the inoculants listed (Table 2.3) are recommended for *in situ* bioremediation (soil and groundwater cleanup, landfarming).

2.4 Considerations in the Use of Commercial Inoculants

Vendors of current commercial inoculants cite a number of benefits of their products, mostly stemming from convenience and time constraints. The most important benefit of their inoculants is efficiency. Through intentional culturing, characterization and blending, commercial inoculant blends can be optimized in advance for high efficiency. Individual blends can also be custom tailored for specific environments and contaminant exposures. This should ensure that adaptation periods are small and activity is optimum.

In addition, the nutritional and physiological needs (*i.e.*, pH, temperature) of the degrader-microorganisms are, or should be, well defined. The bacteria can then be added in large quantities with relatively short notice, and can be replenished according to set schedules.
Closer scrutiny of these benefits, however, reveals significant limitations of commercial inoculants for enhancement of *in situ* and on-site bioremediation. A number of researchers contacted through the inoculant survey raised concerns over the efficacy of microbial inoculants and over public and environmental health. These concerns and considerations in the use of commercia inoculants are discussed below.

2.4.1 Efficacy

The question of efficacy of microbial inoculants is largely based on theoretical considerations of microbial ecology. Existing microbial communities tend to represent climax ecological communities which have survived natural selection and are thus best suited to occupy their niche. As such, these communities often exhibit the property of homeostasis and are quantitatively and qualitatively stable when subjected to moderate levels of biotic or abiotic stress (Suflita, 1989a). It is ecologically unreasonable to expect competitive success from inoculants produced without competition in the laboratory and then forced into *in situ* competition, unless the inoculants have been subjected to a rigorous research program designed to select for competitive strains (Trevors, pers. comm.).

Dr. Ryan Dupont (pers. comm.) and the Utah Water Research Laboratory have undertaken several experiments dealing with modifications of contaminated environments to stimulate indigenous microbial populations. None of the studies relied on the use of exogenous microorganisms and none were limited by the indigenous populations available from the native soils. He states, "Our experience has made us quite sceptical of claims of microbial amendment vendors, or of the need

for that matter for adding organisms to natural environments. Questions arise as to the ability of lab-cultured, substrate specific organisms to survive fierce competition from indigenous cultures which have evolved under natural conditions".

The fierce competition between allochthonous and indigenous microorganisms referred to by Dupont is for available niches, space (surfaces), microhabitats and nutrients. This competition will occur *in situ* and in on-site treatment systems (*e.g.*, reactors) in which microbial inocula is added to contaminated soil or water containing indigenous microorganisms. Successful establishment of an inoculant will be greater in soils and waters that have had the indigenous microbial population reduced or eradicated. The potential to successfully inoculate contaminated soils and waters will increase in on-site systems that pre-treat the soil or water to reduce the population density of indigenous microorganisms. The degree of reduction of the indigenous populations will be a function of the carrying capacity of the particular soil and water, and the effectiveness of the remaining population to colonize as compared to the allochthonous microbes.

Predation on the microbial population by protozoans also negatively impacts on the success of allochthonous microbes. Dr. Tom Phelps (pers. comm.) states that the use of inoculants should be approached with caution as protozoa rapidly graze upon allochthonous microorganisms. Rapid removal by predation of the allochthonous microorganisms occurs because they are introduced in high numbers and cannot move into protected microhabitats faster than the grazing rate of the protozoans. Phelps also discussed how several of his experimental bioreactors were quickly colonized by protozoans that are naturally present in groundwaters.

An example of the survival of allochthonous microorganisms is provided in Table 2.6. This table shows that in all but on case, allochthonous microorganisms introduced in manured and limed soils failed to survive significantly beyond 45 days after inoculation. The success of allochthonous microorganisms, particularly in oligotrophic environments, would likely follow similar trends.

The "fitness" of a microbial inoculant may also influence its success. Often the manner in which the laboratory cultures are grown and/or stored significantly affect their subsequent survival and activity in the open environment (Trevors, pers com.). For example, microorganisms grown in nutrient rich conditions may not be metabolically poised for the nutrient poor (*i.e.*, oligotrophic) conditions present in many soils and waters.

Cell survival and activity of lab-cultured strains in the environment can however, be extended by improving the specific formulations of the products (Trevors, pers. comm.). For example, microorganisms introduced into nutrient poor environments may "leak" essential minerals such as magnesium (required for stabilization of ribosomes, membranes, nucleic acids) and potassium (required to activate many enzymes) because of membrane damage. Growing the microorganisms in a media containing elevated levels of magnesium and potassium, may enhance the survival and activity of introduced microorganisms. The use of an appropriate carrier may also extend the survival of allochthonous microorganisms in novel settings (Trevors, 1991b). Carriers may increase survival by creating an additional niche within which the introduced microorganism can colonize and survive (*e.g.*, peat, alignate, woodchips). Carriers may also ensure that key or unique nutrients are in close proximity to the introduced microbes, thus promoting successful establishment and colonization of the surrounding habitats. Additional research is needed in these areas.

A major limitation to the *in situ* use of allochthonous microorganisms is that it is difficult to ensure their distribution within soils and groundwaters due to factors such as adsorption, survival, filtration, and predation (Pekdeger and Matthess, 1983, Matthess and Pekdeger, 1985; Phelps, pers com.). The problem of adequate distribution is of less concern in engineered systems such as bioreactors or biopiles because the soil and water can be mechanically mixed.

In general, our understanding of how microorganisms survive, interact and function in competitive environments is limited. Often, emphasis has been placed on what chemicals a particular species of microorganism can biodegrade, or how can we modify the organisms to obtain desired catabolic activity(ies). Too little effort has been spent assessing first what makes a microorganism a good competitor in a given environment, and then engineering the environment to favour the desired microorganism. Combining effective competitors and a favourably engineered environment will likely increase the success of bioremediation.

Another concern over the use of inoculants is the ability to add sufficient biomass, notably *in situ*, to initiate activity and achieve desired results. Dr. Barbara Hemmingsen (pers. comm.) believes that it is, in principle, nearly impossible to add enough laboratory grown bacteria to the soil to make a difference, especially since such bacteria probably can't compete with the existing 10^6 to 10^{10} indigenous bacteria per mL or per gram of soil (dry weight). The cost of such additions, to levels causing notable effects (50% of the total population) could make bioremediation unfeasible. Situations may however exist whereby a unique microbial strain

emerges from a sound scientific research program and proves to be a good competitor under certain environmental circumstances (Trevors, pers comm).

Merkert Laboratories (promotional brochure) and Polybac Corporation (Magniarini, pers. comm.) defend the use of inoculants by indicating that it is not the intention of vendors to provide a ready made population which will immediately digest contaminants but rather their intention is to apply a sufficient amount of product to give an initial population of 10^6 microorganisms per gram of soil. It is their belief that this concentration ensures a sufficient population will be provided for rapid bacterial growth. However, this "rapid microbial growth" depends upon the ability of added bacteria to survive and out-compete the indigenous microorganisms during imposed changes in nutrient and physicochemical conditions. Even if the surviving allochthonous microbes can be selectively enhanced to grow faster than the indigenous population, acceptable biodegradation rates may not ensue as the allochthonous microorganisms may utilize other non-target compounds (see section 3.2.2.6) for their growth and survival.

In comparing bio-augmentation with natural degradation (biostimulation), Merkert Laboratories feels the former greatly increases the rate of degradation. While microorganisms naturally present can degrade contaminants, they are often present only in the upper few centimetres of soil and their cell density is low. These organisms are greatly diluted when mixed with a large amount of excavated soil. The dilution factor can be so large that it takes an excessively long time, if ever, to establish a working population of appropriate microorganisms. This rational does not agree with research conducted over the past 15 years which indicates that a substantial population of active microorganisms (10^6 microorganisms per gram dry weight of soil) exist to depths of over 1,500 feet (Phelps *et al.*, 1989; Sulflita, 1989a).

In general, it is difficult to assess claims by microbial inoculant vendors as they do not monitor for the microorganisms they introduce, nor do they check background populations to determine if their microorganisms are already present at the site. It is thus difficult to confirm if allochthonous or indigenous microorganisms were responsible for observed biotransformation and/or reduction in toxicity.

Several organizations (OHM Corporation, ADS Associes, CET/Thorne Environmental) have undertaken bioremediation using indigenous microorganisms. Mr. Paul Flatham of OHM Corporation (pers. comm.) believes that the microorganisms in commercial inoculants are present in most natural environments, and in most cases, the desired indigenous microorganisms can be

stimulated to undertake bioremediation. Table 2.7 provides a list of microorganisms that have been detected in or isolated from contaminated soil and/or groundwater. When compared with the contents of current commercial inoculants, it is evident that very few microorganisms used in microbial inoculants are not present in natural and/or contaminated environments. This underlines the need for *a priori* characterization of the indigenous microbial community structure (section 3.3.2) and microbial activity (3.3.3) to ensure the need for an inoculant.

Perhaps the major factor limiting acceptance of commercial inoculants is the lack of conclusive proof, based on efficacy, that the use of such inoculants constitutes wise scientific and financial practice. Dr. Barbara Hemmingsen (pers. comm.) has received and tested the efficacy of allochthonous microorganisms from three different producers and she states "it is clear that their addition did not noticeably affect the rate of disappearance of the full hydrocarbon from the soil." Hemmingsen goes on to indicate that the commercial preparations used contained only 0.01-0.001% of their weight as bacteria, and in two out of three cases none of the bacteria present could degrade benzene, toluene, xylenes or phenanthrene (which the commercial producers claim their inoculants could degrade). Misleading claims regarding bioremediation obviously plague the acceptance of commercial inoculants. The use or abuse of such claims could perhaps be reduced by implementation of efficacy and quality control regulations.

The key for acceptance of inoculants for widespread use seems to be provision of proof that these products contain live, healthy bacteria of the proper physiological make-up and that such bacteria can compete with native bacteria to effect the breakdown of a pollutant. Proof will require replicated field trials using proper statistical analysis, evaluation protocols and proper controls. Dupont states that none of the case studies he has reviewed have actually shown the effectiveness of allochthonous microorganisms because the studies suffer from a lack of even simple controls (side by side plots with nutrient solution but no organisms added), and even studies from what he considered reputable firms are poorly conceived and executed.

2.4.2 Environmental and Health Concerns

The use of commercial inoculants also raises important environmental and public health concerns. Natural strains may be undesirable plant or animal pathogens or may produce toxins (see Table 2.4). Identification and evaluation of the contents of microbial inoculants (notably mixtures of microorganisms) will be important to prevent the release of undesirable microorganisms into the

environment and to ensure proper safety during manufacturing and proper disposal of by-products of the manufacturing process.

Identification of culture composition will also be crucial for quality control (*i.e.*, assuring the consistency of the microbial inoculant composition and for *post priori* analysis of why a particular bioremediation effort fails). Lack of ability to track and monitor released microorganisms will make it virtually impossible to determine the cause of failure of a bioremediation venture.

Identification of microorganisms in inoculants also assists in identifying strains which may carry antibiotic resistance on transmissible plasmid DNA. This would be undesirable from a public health perspective as it may introduce antibiotic resistance into the gene pool of a given open environment. Proper identification and characterization of all microorganisms included in commercial inoculants would allow detection of potentially non-desirable traits and would prevent release of undesirable microorganisms.

Genetic drift and spontaneous mutation (deletions) may also be experienced by microorganisms released into the environment. The very nature of the open environment with transducing phages, plasmids and transposons present suggests that some amount of gene transfer will occur. The frequency of such transfer may be relatively low (10^{-8}) or virtually undetectable, but the risk associated with transfer of catabolic genes should be addressed. These problems should be identified at the research and development stage, possibly by microcosm and/or small-scale field testing. While it is difficult to assess mutation and gene transfer events, it will be important because deletion or transfer of a gene essential for biodegradation of a targeted compound may cause failure of bioremediation.

2.5 Future Direction in Commercial Inoculants

Three active research areas have been identified by various researchers are:

- isolation of additional naturally occurring microorganisms capable of biodegrading targeted pollutants;
- improvement of existing and novel microorganisms to enhance their competitive and catabolic ability in natural environments. Enhancement may include improving the characteristics of the microorganisms through genetic engineering

and selection or by identifying and controlling the factors that affect their survival and activity; and

- development of non-aqueous microbial formulations and carriers which increase the stability and success of introduced microorganisms (see section 2.4.1).

There is a need to isolate and properly identify more novel indigenous microbial strains from contaminated sites and assess their value for the biodegradation of targeted pollutants. Rigorous scientific study of new strains may result in the emergence of a microbial strain of excellent value in bioremediation. Examining the genetic and molecular aspects of catabolic genes and the physiology of existing and newly isolated microorganisms, and studying the ecology of microorganisms (through microcosms and small-scale field trials) will be essential in future bioremediation research. An emphasis will also likely be placed on the use of radiolabelled tracer studies and on regulated controls and treatments in both microcosms and small-scale field trials. Biodegradation rates are often relatively slow in the open environment, supporting the need for better designed feasibility studies which allow extrapolation of laboratory results to the field (Alexander, 1985; Trevors, pers. comm).

Genetically engineered microorganisms (GEMs) are currently being developed for use in bioremediation however, regulatory approval for use of GEMs will be more difficult to obtain than approval for use of naturally occurring microorganisms. Indigenous strains are considered to be less harmful than GEMs from an environmental and public health perspective. However, genetic engineering of strains may potentially be useful as it will eliminate many problems discussed in section 2.4 (*i.e.*, use of pathogenic microorganisms and microorganisms harbouring antibiotic resistances and/or transmissible plasmids could be avoided).

GEMs may be safer from both environmental and public health perspectives as data available for these strains is likely extensive if the research has been comprehensive. It is likely that GEMs will find their first application in bioreactors in which their release can be closely monitored and controlled.

Through genetic engineering, microorganisms with appropriate survival abilities could receive catabolic genes. Table 2.8 lists a number of microorganisms, mostly pseudomonads due to their ecological fitness, which have received genes encoding novel catabolism. The use of these GEMs in bioremediation will depend on successful expression of the gene(s) in the environment.

Expression of gene products may be low in the natural environment and it is possible that the targeted pollutants will not enter the GEM, thereby limiting the rate of biodegradation.

The use of GEMs will also depend on the stability of inserted genes in the environment. For example, genes located on plasmids or transposons (mobile genetic elements) may be lost from cells attempting to reduce their metabolic load and as such, the desired metabolic activity may be lost. Alternatively, the inserted gene may be transferred to indigenous microorganisms, thereby providing indigenous microbes with a potentially undesirable attribute. These problems would hopefully be identified at the microcosm testing stage in the laboratory, and/or during small-scale field trials.

3.0 IDENTIFICATION OF INFORMATION REQUIREMENTS

The purpose of this section is to present and discuss the factors that influence bioremediation. These factors were culled from responding researchers (Table 3.1) and the available literature (Table 3.2). The theoretical background of the suggested information requirements and the rational for their inclusion are discussed to provide the reader with an understanding of how to evaluate and assess bioremediation. **It is important to note that not all the suggested information requirements are needed to evaluate bioremediation. The required information and criteria will be case specific (*i.e.*, site specific).** Section 4.0 indicates how to use and combine information to properly evaluate and validate bioremediation.

The reader is advised that many information requirements are highly interrelated and thus they should not be considered in isolation. It may be possible to infer, or fill in, missing information, or to "logically link indirect evidence" to come to a conclusion on the feasibility or success of bioremediation (Madsen, 1991b). This will be particularly true in cases where collection of all suggested information is not feasible or when it is not possible to obtain or estimate realistic values for the information requirements identified.

Where possible, criteria (*e.g.*, ranges of values such as pH) are provided within which bioremediation is more likely to proceed. This is not to say that bioremediation is not feasible, or is not occurring, if the criteria are not met or if measured values are outside ideal ranges. Only by examining the available information in its entirety can a particular bioremediation proposal or application be evaluated.

The information requirements are used for two reasons; 1) evaluating the feasibility of bioremediation; and 2) monitoring to validate bioremediation. The majority of the suggested information requirements can be used for both feasibility and validation purposes, but several information requirements will be used exclusively for validation or feasibility evaluation. The appropriate use of each information requirement is noted.

Similarly, the suggested information requirements have been generalized to be applicable to both *in situ* and on-site bioremediation. However, whereas all of the suggested information requirements are applicable to *in situ* bioremediation, only a subset are applicable to on-site bioremediation. We believe that this subset covers the information requirements for on-site

bioremediation. Appropriate information requirements for on-site versus *in situ* bioremediation are noted.

The suggested information requirements are divided into three main categories, which are;

- contaminant(s), Section 3.1;
- site physicochemical parameters, Section 3.2; and
- microbial characterization, Section 3.3.

A brief overview of each suggested information requirement is provided below.

3.1 Contaminant Characterization

The nature of a contaminant may dictate if its biodegradation is possible, and if so the rate of degradation. Information requirements for contaminant characterization are subdivided into three key sub-categories;

- identification of chemical attributes of the contaminant(s)
- biodegradability; and
- characterization of contaminant distribution.

3.1.1 Contaminant Attributes

Many chemicals have been documented to be biotransformed by various microorganisms (see Table 2.5). Generally, if the published scientific literature indicates that a specific chemical or class of compounds can be biodegraded under both laboratory and/or "real" environmental conditions, the greater confidence there is that a given chemical can be biodegraded at a new site, given other conditions are met (section 3.2.1, 3.2.2). For example, biodegradation of the gasoline components benzene, toluene, and xylenes (referred to as BTX) and simple straight chain hydrocarbons has been well documented in terms of the metabolic pathways, microorganisms and environmental conditions involved or required. Thus, there will be greater confidence in successfully bioremediating sites contaminated with BTX.

However, many chemicals exist for which there is either little or no available information on if or how they are metabolized. In these cases, it may be possible to initially assess the potential

of biodegradation by examining various chemical attributes. Information on chemical structure (section 3.1.1.1), solubility (section 3.1.1.2), volatilization (section 3.1.1.3), sorption (section 3.1.1.4), and chemical reactions (section 3.1.1.5) serves to assess contaminant biodegradability for bioremediation evaluation and will also serve to assess biodegradation data provided during monitoring programs.

3.1.1.1 Chemical Structure

Certain chemicals are inherently more susceptible to microbial degradation than others owing to differences in structure, chain length, molecular weight, types of chemical bonds, branching, and substituents. Each of these factors may affect the ability of a microorganism to metabolize a compound and obtain energy from the process, and as such these factors may influence the success of bioremediation. Biodegradability of chemical structures can be somewhat predicted based upon following trends;

- The rate of biodegradation decreases with increasing molecular weight (and decreasing solubility). For example, hydrocarbon liquids (chain length of 10 to 13 carbon atoms) are attacked by a large number of microorganisms while viscous and/or solid hydrocarbons (chain length of 19-24 carbon atoms) are very insoluble and are not susceptible to microbial attack under normal temperatures (Rittman *et al.*, 1991);

- Alcohols, amines, esters, mercaptans, carboxylic acids and nitriles are usually biodegradable but may accumulate to toxic levels. Nitro substitutions and ether linkages generally increase resistance to biodegradation;

- Branching of straight chain contaminants (*i.e.*, cutting oils) increases recalcitrance;

- Aromatic compounds with up to two rings (benzene, naphthalene) are often biodegradable. Recalcitrance increases as the number of rings increases;

- Increasing the number of substituents (*i.e.*, NO_2, OH, X) on a ring generally increases recalcitrance (see Table 3.3);

- The type and position of a substituent will influence the rate of contaminant transformation (*i.e.*, dehalogenation, Table 3.4).

- Increasing halogenation (addition of chlorine is most common) generally increases recalcitrance to aerobic biotransformation but decreases recalcitrance under anaerobic conditions (*i.e.*, reductive dechlorination by anaerobic microorganisms such as methanogens).

Identification of the structural attributes of the contaminant(s) will allow predictions to be made as to the potential recalcitrance of the contaminant(s) of concern. When coupled with identification of the biodegradation pathways (section 3.1.2.1), the microbial communities (section 3.3.2) and geochemical (section 3.2.1) conditions, identification of the structural attributes should allow an educated guess as to the fate of the contaminant(s). If there is indication that biodegradation is feasible, then it is justifiable to conduct treatability studies (Section 4.0).

3.1.1.2 Solubility

In general, there is a direct relationship between solubility and biodegradation rate. Biodegradation requires that cells possess a means of transporting substrates (contaminants) across the cell envelope to the intracellular site of catabolism (Rittman *et al*, 1990). Mass transfer of substrates occurs predominantly through uptake of contaminants dissolved in the aqueous phase. Thus, the more soluble a contaminant is, the more likely it is to be transported into the cell and metabolized. Kobus and Kinzelbach (1989) attributed accelerated microbial activity and successful bioremediation to the complete dissolution of the organic contaminants (hydrocarbons) into the aqueous phase (groundwater), which guaranteed complete substrate availability. Thus, the solubility of the contaminant(s) to be bioremediated should be identified *a priori* to determine the potential availability of the contaminant(s) for biodegradation. Table 3.5 provides solubility values for a number of common organic contaminants.

The transfer of substrates into cells can also occur through direct contact, depending on the size of the molecule. For large molecules (*e.g.*, hydrocarbon chains) addition of emulsifying agents

may be required to effect Pseudosolubilization. This will increase the surface area of the contaminant and may increase its solubility, possibly leading to its biodegradation. Likewise, the addition of biological or chemical surfactants may increase solubility. The use of organic biodegradable surfactants should however be approached with caution as microbial production of gas and biomass may lead to clogging of the treatment system (Kobus and Kinzelbach, 1989).

Polymers and other large, complex, insoluble compounds which cannot be transported across cell membranes are often not biodegraded. Certain microorganisms, however, can degrade these otherwise recalcitrant polymers by secreting exoenzymes which cleave the polymer into smaller, more soluble components which can be taken up and metabolized.

The solubility of most contaminants can be derived from current literature and, if necessary, solubility values can be obtained from experimental quantification. The effect of contaminant solubility on microbial biodegradation rates can be determined by treatability studies (*i.e.*, microcosm, section 4.0).

Monitoring the solubility of hydrophobic (low solubility) contaminant(s) during bioremediation may provide an indication of increased bioactivity and may provide indirect evidence of bioremediation. For example, many microorganisms release emulsifying agents which can increase the solubility of contaminants and thus increase their availability. Lee *et al.* (1988) isolated a *Pseudomonas* species that produced an emulsifying agent in the presence of PAHs, resulting in increased PAH solubility and thus increased aqueous concentrations of PAHs.

3.1.1.3 Volatilization

Volatilization is the rapid transfer of a molecule from an aqueous solution to the vapour phase (phase transfer). It is an abiotic factor which is often responsible for loss of contaminant mass. The volatilization of a contaminant will depend on its Henry's Law Constant and thus the potential for mass transfer or loss through volatilization will be dependant on the contaminant(s) involved. Table 3.5 provides Henry's Constants for a number of common organic contaminants. In general, as the Henry's constant increases, the potential for volatilization increases.

Monitoring activities should consider volatilization for several reasons. Firstly, migration of volatile contaminants may result in their redistribution and may increase the overall extent of contamination. Secondly, bioremediation treatment systems may promote volatilization and thus

mass loss of the contaminant. The volatilized contaminant should be accounted for to determine the amount of mass loss that can be attributed to biodegradation. Ideally, the volatilized contaminant should be treated either by recirculation through the biotreatment system or by an alternate technology.

3.1.1.4 Sorption

It is not coincidence that many compounds which are persistent in the environment are highly hydrophobic and water-insoluble, and are therefore present in the aqueous phase at very low concentrations while present at high total concentrations (Stroo, unpubl.). The effect of sorption on biodegradation is quite complex. Results of several studies (van Loosdrecht *et al.*, 1990; Ogram *et al.*, 1985; Breznak *et al.*, 1984) suggest that sorption of substrates can cause both a stimulation or a retardation of biodegradation.

Sorption can negatively affect biodegradation by several means. Sorption of a contaminant to subsurface materials results in decreased concentrations of that contaminant in solution. If the substrate utilized by bacteria is strongly but reversibly sorbed (primarily hydrophobic), the substrate biodegradation rate will be limited by the rate of desorption and thus the biodegradation rate will be a function of the solid surface area impacted (Rittman *et al.*, 1991). Irreversible sorption of the substrate would ultimately result in inhibition of its biodegradation. Stieber *et al.* (1990) found that clay soils had a high capacity of adsorption to PAHs, such that removal and biodegradation of the PAHs from the soil was less successful than expected.

The influence of sorption on biodegradation may be positive in cases where substrate concentrations are toxic to microorganisms. Sorption of the substrate may decrease aqueous concentrations such that microbial degradation is not limited by toxicity. As the substrate is biodegraded and its concentration declines, kinetics will determine desorption of the sorbed substrate, thereby making the substrate available at a rate and concentration at which the microorganism(s) can survive and function (Rittman *et al.*, 1991). These effects have been described as buffer and reservoir actions.

An experiment by Ehrhardt and Rehm (1985) demonstrated the potential positive influence of sorption. They found that cells in solution could not grow with phenol concentrations greater than 1.5 g/L however, in the presence of activated carbon, cells could tolerate phenol concentrations up to 15 g/L, and could degrade 90% of the adsorbed phenol. Experiments by

Apajalahti and Salkinoja-Salonen (1984) found that the addition of bark chips to their treatment system promoted PCP degradation by reversibly sorbing PCP and removing toxicity of high PCP concentrations.

The potential for sorption to influence bioremediation must be identified to ensure maximization of the overall rate and efficiency of bioremediation. Manipulation of the sorptive capacity of a system (through surfactants and/or addition of organic matter) may be beneficial to maintain optimum aqueous contaminant concentrations. Manipulation of sorption during on-site surface applications (*e.g.*, reactors, composting) through mixing and addition of surfactants or emulsifiers will be relatively easy while manipulation of *in situ* environments to create proper buffer/reservoir conditions will become increasingly difficult and expensive with depth and decreasing permeability.

The potential for sorption to control biodegradation should dictate that evaluation of bioremediation should not be solely based on aqueous concentrations of the contaminant(s). A significant amount of the contaminant may be bound to soil/solid surfaces and must be accounted for, particularly with respect to mass balancing. The influence of sorption/desorption on contaminant concentration should be assessed to ensure that microbial activity will not be limited by contaminant availability or toxicity.

Table 3.5 provides log K_{ow} values for some common organic contaminants. These values are based upon the hydrophobicity of the chemicals, with lower values indicating lower potential for sorption.

3.1.1.5 Chemical Reactions

Changes in water chemistry may occur as a result of inorganic reactions rather than biological reactions. It will be important to identify and quantify these reactions as they may influence successful evaluation and monitoring of bioremediation. Reactions to consider with respect to the contaminant include hydrolysis, photooxidation, and reduction. Precipitation reactions should also be considered for added nutrients.

Of major concern will be the reaction of nutrients, such as nitrates and phosphates. The geochemistry of subsurface waters may cause precipitation of such nutrients as insoluble salts, thereby removing the nutrients from microbial use. The success of a bioremediation strategy may

then be limited by the ability to successfully distribute nutrients to the subsurface microorganisms. Feasibility of applying bioremediation should consider if the site geochemistry will adversely affect the delivery of the nutrients, and if so, if the geochemistry be modified to allow proper nutrient distribution. Conversely, monitoring programs should consider the possible influence of geochemical changes during bioremediation which may adversely affect the delivery of nutrients and hence bioremediation.

Abiotic degradation of contaminants should also be evaluated to determine if bioremediation is required. For example, abiotic chemical reactions may remove contaminants at a greater rate than bioremediation. Table 3.6 provides a comparison of general chemical and biological degradation rates for some common organic contaminants.

Validating bioremediation should identify and quantify abiotic degradation reactions to assess the amount of mass loss due to abiotic versus biotic mechanisms.

3.1.2 Biodegradability

Familiarity with which compounds or classes of compounds are biodegradable, the metabolic pathways and the conditions under which compounds are biodegraded, will assist in evaluating the feasibility of bioremediation. This knowledge should also assist in determining which organic/inorganic, physical and other parameters should be monitored. If this knowledge is lacking or incomplete, treatability studies (section 4.0) should be undertaken.

The extant information, while of significant value, should not be used as a substitute for treatability studies but should be used in designing and determining the effort level of treatability studies. For example, toluene has been well documented to be biodegraded to carbon dioxide (CO_2) under both aerobic and denitrifying conditions. Thus, a laboratory treatability study in which the production of CO_2 from toluene was measured, and mass balance was observed, may be sufficient to be confident that toluene degradation would occur at the site. However, there is a paucity of data on the biodegradation of halogenated compounds such as Freon. In these cases, radiolabelled treatability studies may be required to define the biochemical pathways involved. This information will be useful in determining which bioremediation strategies will be appropriate and which geochemical manipulations may be required to induce the appropriate biochemical pathway(s). Radiolabel treatability studies will also allow identification of the intermediates and end products that will be produced and will thus assist in ensuring that

biochemical pathways yielding recalcitrant and/or toxic metabolites are not chosen (see section 4.1.2). Identification of these metabolites will also help in designing appropriate monitoring programs.

3.1.2.1 Biodegradation of Classes of Compounds

Table 3.7 provides a brief list of the biodegradation pathways and metabolites common to several major classes of chemicals. For a more complete review, the reader is referred to Rittman *et al.* (1991).

Identification and characterization of the pathway(s) by which contaminants will be biodegraded will allow proper identification of the metabolic intermediates and end products that will be produced during bioremediation. This will be key to both evaluating bioremediation strategies and designing appropriate monitoring programs for bioremediation. A review of the chemical attributes and literature of these metabolites will ensure the proposed biodegradation pathway(s) do not yield more toxic or more recalcitrant compounds.

Biodegradation will involve transformations in the structure of the contaminant(s) being metabolized. Such transformations may be detectable during monitoring of bioremediation. For example, in an anaerobic environment, reductive dechlorination of trichloroethene will result in the production of lower chlorinated aliphatics. The detection of dechlorinated products can be used as indirect evidence of biodegradation.

Monitoring programs should be tailored to include detection of the expected metabolic intermediates and end products. Detection of daughter (intermediate) products will serve to confirm if predicted microbial pathways are being used and if expected metabolites are being produced in the field. Some metabolites and end products are unstable or are not accumulated and hence, their detection may be difficult. For example, complete biodegradation (mineralization) will produce end products of carbon dioxide and water, origins of which will be difficult to trace without proper labelling techniques (*i.e.*, radiolabelling techniques, section 4.1.2).

It is important to recognize if the contaminant supports the growth of the microorganism(s) or if it is biodegraded indirectly (see section 3.1.2.2). The difference is important in evaluating the laboratory and field microbial biomass growth data.

3.1.2.2 Metabolic Processes for Biodegradation of Recalcitrant Contaminants

Contaminants can be "recognized" and directly metabolized for carbon, energy or for key nutrients by microorganisms. However, many compounds which do not directly support the growth of microorganisms are still biodegraded. This apparent contradiction lies in the fact that many contaminants are structurally similar to natural compounds, and if the appropriate enzymes are active they can completely or partially react with the contaminant. Metabolic strategies presented below include analog degradation and cometabolism. Both of these processes usually require interactions among various species or physiological function groups of the microbial population to completely detoxify or mineralize the pollutant. As a result, the population of the microorganism(s) which are key in the biodegradation may not actually increase in size, whereas the general microbial population size may increase because the initial transformation of the contaminant may yield utilizable substrate(s) for microbial growth.

Analog Degradation: Analog degradation is an indirect method by which contaminants can be metabolized. Certain microorganisms possess enzymes which have a high specificity with respect to their catalytic function, but low specificity with respect to substrate binding. The functional groups of certain molecules (*i.e.*, contaminants) may cause induction of enzyme systems, leading to production of enzymes which indirectly transform certain analog molecules. Analog degradation is a method whereby microorganisms can be induced to biotransform a compound which is otherwise resistant to degradation.

Several examples of analog degradation exist, however, few have been researched with regards to the degradation of contaminants in the environment. A number of microorganisms (*e.g.*, *Azotobacter*, *Rhizobium*, *Nitrobacter*) can use inert nitrogen gas as a nitrogen source. This ability is provided by the nitrogenase enzyme which splits the triple bond between the nitrogen atoms. When active, this enzyme also incidently transforms acetylene, a structural analog of N_2. Additional examples of analog degradation include: the degradation of dibenzo(p)dioxins by enzymes produced by PAH-degrading *Phanerochaete chrysosporium* (Hammel *et al.*, 1986); the degradation of *p*-xylene by enzymes produced by toluene-degrading microorganisms (Alvarez and Vogel, 1991).

In cases where analog biodegradation will be used for bioremediation of contaminants, the particular analog mechanism (*i.e.*, the primary substrate, the enzyme involved and the analog) should be identified and the ability of the analog to induce enzyme synthesis and activity should

be demonstrated. Metabolites produced from both the primary substrate and the analog must also be identified and the toxicity of the metabolites and the initial substrates must be addressed using standard bioassay techniques. Analog degradation as a biodegradative tool may be limited because the structurally similar compound that must be present to induce enzyme synthesis is likely a hazardous pollutant itself (Rittman *et al.*, 1991).

Successful analog degradation will result in a reduction of the targeted contaminant coupled with the production of identifiable metabolites. Monitoring programs should be designed to include analysis for these metabolites. Detection of these metabolites, coupled with reduction of the target contaminant will provide evidence of bioremediation. Successful analog degradation should also result in the production of increased levels of the induced enzyme. Assays for this enzyme would provide indirect support to validate bioremediation.

Cometabolism: Cometabolism is another method of indirect substrate metabolism. In the case of cometabolism microorganisms actively metabolize a dissolved organic for energy and carbon while biotransforming another dissolved organic which fits an active enzymatic site. This transformation provides no gain of energy, carbon or any other nutrient for the microorganism responsible, however, products released may be susceptible to further microbial attack and may be used as energy and carbon sources by other microorganisms.

An example of cometabolism as an important strategy for bioremediation is the oxidation of chlorinated aliphatics by methanotrophic bacteria (Henson *et al.*, 1988; Wilson and White, 1986; Wackett and Gibson, 1988; Wackett *et al.*, 1989; Oldenhuis *et al.*, 1989; Semprini *et al.*, 1988). Wilson and Wilson (1985) documented the cometabolism of TCE to CO_2 by methanotrophs utilizing natural gas as a primary carbon and energy source. A number of pesticides have also been shown to be cometabolized. For example, Horvath (1971a,b) demonstrated the cometabolism of 2,3,6-trichlorobenzoate and 2,4,5-trichlorophenoxyacetic acid by both *Brevibacterium* spp. and *Achromobacter* spp. Pfaender and Alexander (1973) have demonstrated the cometabolism of DDT by microorganisms in raw sewage.

Co-metabolism can be a very useful method of transforming contaminants without interference of threshold limitations (see S_{min}, section 3.1.2.3) however, co-metabolism may result in the conversion of certain compounds into more toxic intermediates (*i.e.*, TCE to vinyl chloride). Co-metabolism as a biodegradative tool is also limited because the structurally similar compound that

must be present to induce enzyme activity tends to be a hazardous pollutant itself (Rittman *et al.*, 1991).

In cases where cometabolism will be used for bioremediation of contaminants, the particular cometabolism mechanism (*i.e.*, both the primary substrate, the enzyme involved and the cometabolite) should be identified and demonstrated by appropriate treatability studies. Identification of the possible metabolites produced by both the primary substrate and the cometabolite will be required as will information on the toxicity of the metabolites and the initial substrates.

Successful cometabolism will result in a reduction of the primary substrate and the targeted contaminant and in the production of identifiable metabolites. Monitoring programs should be designed to include analysis for these metabolites. Detection of these metabolites, coupled with reduction of the cometabolite and the primary substrate will provide evidence of bioremediation.

3.1.2.3 Concepts of S_{min}, Non-steady State Growth and Secondary Substrate Utilization

Although many microorganisms can use organic compounds as the sole source of energy and carbon, threshold concentrations (referred to as S_{min}) exist below which growth, activity and maintenance of cell requirements is not possible (Alexander, 1985). S_{min} is defined as the minimum concentration of a substrate (*i.e.*, contaminant) which can support steady-state growth, given that all required nutrients are in excess except the limiting substrate, termed S.

Monod kinetics for bacterial growth in biofilms and suspended growth cultures can be used to predict and/or calculate the S_{min} of a substrate. The fundamental equations for determination of S_{min} will not be provided herein as they are extensively reviewed by Rittman *et al.* (1980), Rittman and Brunner (1984), Rittman *et al.* (1986), and Rittman and McCarty (1981a,b). For a brief but concise review of the theoretical calculation of S_{min}, the reader is referred to Rittman *et al.* (1991).

The S_{min} of a substrate can also be determined experimentally during treatability studies using continuous culture techniques (*e.g.*, chemostats) or microcosms. The chemostat is a self-regulating system that reaches a steady-state condition. Changes in the input concentration of the growth limiting substrate produces changes in the concentrations of microorganisms

detectable in the washout rate. When the concentration is below the S_{min} of the substrate, the microorganisms will not be able to maintain steady-state growth and net washout will be observed. The S_{min} can also be determined in static microcosms by determining the concentration of substrate that cannot be assimilated by microorganisms.

S_{min} will be an important consideration in the evaluation of bioremediation. For example, if the S_{min} of a contaminant exceeds the regulatory limit (*i.e.*, S_{min} = 10 ug/L; regulatory limit = 1.0 ug/L), then bioremediation may not degrade the contaminant to concentrations below the regulatory limit. In this case, bioremediation would not be an effective remediation option unless a cost effective polishing step (*i.e.*, carbon adsorption) could be coupled to the biotreatment to achieve cleanup goals. Sites with initial contaminant concentrations lower than the S_{min} of the contaminant may also not be candidates for bioremediation as steady-state growth would not be achieved and catabolism would not occur.

Problems associated with threshold concentrations (S_{min}) can be circumvented by either non-steady state substrate utilization or secondary substrate utilization. Non-steady state substrate utilization requires that biomass undergoes net decay while still utilizing the concentrations of the substrate below its S_{min}. Thus, non-steady state substrate utilization may be an effective means of reducing contaminant concentration below regulatory limits in cases where significant biomass exists or can be introduced. Non-steady-state substrate utilization will not be an effective method of initiating contaminant removal in cases where initial biomass is low.

It is also conceivable that contaminants present at threshold-level concentrations can be utilized as a secondary substrates. In this process, microorganisms derive most, but not all, of their carbon and energy from other primary compounds, and supplement their energy and carbon shortfall by utilizing the contaminant. Thus the contaminant serves as a source of energy and carbon, but is not the primary source of energy or carbon. The primary substrate, the electron donor contributing electrons and energy to the long-term growth and maintenance, can be a single compound or can be a combination of compounds or secondary substrates (Rittman *et al.*, 1991).

LaPat-Polasko *et al.* (1984) have demonstrated secondary substrate utilization of methylene chloride by a *Pseudomonas* spp. strain LP. In both batch and continuously fed reactors, the strain was able to metabolize the primary substrate acetate at milligram per litre concentrations while metabolizing the secondary substrate methylene chloride at microgram per litre concentrations.

Threshold concentration values (S_{min}) and minimum allowable toxicity limits (regulatory limits) of contaminants must be compared *a priori* to ensure that a bioremediation strategy will be capable of reducing contaminants below allowable limits. If a contaminant will not be biodegraded below its allowable limit by the proposed application, use of non-steady state or secondary substrate techniques may be required. The use and success of these methods will require evaluation and demonstration to ensure that proper cleanup levels will be met.

It should be noted however, that it may be difficult or impossible to obtain representative S_{min} values for organic chemicals in the environment because of the presence and activities of oligotrophic microorganisms. These microorganisms are highly energy efficient and can scavenge extremely low concentrations of organics, even under poor nutrient conditions. The concept of S_{min} will be most applicable to evaluating the theoretical limits of organic chemical treatment in bioreactors containing well-defined microbial consortia.

3.1.2.4 Kinetics and Biodegradation Rates

Knowledge of the biodegradation rates and kinetics assists in both evaluating cleanup times (reactor retention times) and in monitoring bioremediation processes. Monitoring programs should be designed to include observation and evaluation of the expected biological kinetics (contaminant mass loss and microbial growth) and biodegradation rates based upon initial conditions.

Biodegradation of most organic compounds in the subsurface may be described by monitoring their disappearance (rate of degradation) expressed as a function of concentration. The reliability of the rate determination will be dependant on the accuracy with which abiotic mass losses are delineated. Section 3.1.3.2 discusses problems with interpreting biodegradation in terms of what mass loss can be attributed to known biotic, known abiotic and uncertain factors.

The growth of microorganisms can be similarly described, with the change in biomass expressed as a function of substrate concentration. Here again, the reliability of the estimate is dependant on accurate characterization of other available carbon sources such as natural labile organic compounds (section 3.2.2.6).

It may be possible to predict biodegradation rates and kinetics *a priori*, given the initial biomass size (section 3.3.1) microbial community composition (section 3.3.2) and substrate concentration.

Figure 3.1a categorizes kinetic models as a function of substrate concentration and bacterial cell density. Each model is defined by its order of reaction which is defined as the value of the exponential used to describe the reaction. Zero and first order power rate models are often used in studies as approximations of the degradation of chemicals in soils (Sims, 1990).

Figure 3.1b shows the typical organic substrate disappearance curves associated with the biological kinetic models in Figure 3.1a. Together, these figures show how varying both the size of a microbial population capable of metabolizing a substrate, and the concentration of the substrate affect the observed growth and substrate mass loss kinetics. Factors that influence biodegradation rates and kinetics include sorption (section 3.1.1.4), solubility (section 3.1.1.2) and temperature (section 3.2.1.3).

Laboratory experiments on biodegradation kinetics and rates often employ high concentrations (1-100 µg/ml or g) of target chemicals. Microbial kinetics and transformation rates determined by such experiments cannot always be extrapolated to natural environments where the same organic compounds are encountered at significantly lower concentrations (pg-ng per ml or g) (Alexander, 1985). Many errors have stemmed from the assumption that microorganisms that can metabolize and grow on a substrate at high concentrations can do so at low concentrations and vice-versa. Treatability studies (*i.e.*, microcosm trials, section 4.0) should be used in determining biodegradation kinetics and rates. Key to use of the microcosms is that the test conditions reflect the variety of contaminant concentrations, environmental or biotreatment conditions that are expected to be encountered in the field.

Because the rate and kinetics of biodegradation are affected by factors such as sorption and mass transfer of the contaminants and nutrients, the decision to proceed with bioremediation should not be solely based on how fast the contaminants can be theoretically degraded. For example, Berry-Spark *et al.* (1988) have shown that if all factors limiting biodegradation of benzene, toluene and xylenes are controlled, then biodegradation of BTX in an aquifer can be considered as relatively instantaneous with respect to groundwater flow. Their study modelled both the effect of different biodegradation kinetic and degradation rates, and biomass size on the biodegradation of BTX and generally found that biodegradation was limited by the mass transfer of oxygen into the BTX plume.

3.1.2.5 Toxicity

Microorganisms generally increase their growth rate in response to increasing concentrations of non-inhibitory wastes until a maximum growth rate is achieved (Figure 3.2). Biodegradation of inhibitory compounds, however, may occur at low concentrations but as the substrate concentration increases, the microbial growth rate, and corresponding biodegradation, rate often do not increase (Figure 3.2).

Inhibition of microbial growth and substrate utilization by contaminants can result from several mechanisms depending on the contaminant and on the physiology of the microorganism. Firstly, inhibition can result from interference with specific metabolic processes or enzymes in key metabolic pathways. Toxicants such as cyanide and azide bind to heme iron in cytochromes and prevent electron transfer reactions, thereby limiting a large portion of the energy-generating capability of microbial cells (Rittman *et al.*, 1991). Other contaminants, notably hydrocarbon chains, aromatics and halogenated contaminants, bind to cell surfaces and cause membranes to become leaky. Leaky cells lose their ability to maintain concentration gradients across their membranes, a property key to the generation of cellular energy.

In general, toxicity correlates with the solubility (and availability) of contaminants. The toxicity (and solubility) of aliphatics increases as chain length decreases. Likewise, toxicity of aromatics increases as the aqueous solubility increases (Rittman *et al.*, 1991). This trend seems to reflect the fact that compounds that are more soluble can achieve higher aqueous concentrations and can cause more damage to microbial cells. High concentrations of contaminants may require control during bioremediation by dilution, pumping or by increasing contaminant sorption through addition of materials such as woodchips. Alternatively, certain zones harbouring high contaminant concentrations may require treatment using an alternative technology.

In some cases, toxicity is due to products of microbial metabolism. Bartels *et al.* (1984) showed that toxicity during meta-cleavage of 3-halocatechols was a result of irreversible inhibition of the dioxygenase enzyme by the toxic metabolite acyl halide. Stieber *et al.* (1990) found that biodegradation of PAHs was inhibited by the toxicity of unidentifiable intermediate products, further stressing the need for the identification of metabolites and evaluation of their environmental acceptability.

Perhaps the quickest, simplest and most cost effective method of screening contaminants and expected metabolites for toxicity is the use of bacterial toxicity assays. These assays quantify the toxic effect of a chemical on a test species under specified test conditions (McFarland *et al.*, 1991). Microbial toxicity bioassays may measure the effects of a chemical on overall microbial activity (*e.g.*, respiration, dehydrogenase activity), or on the activity of certain subgroups of the microbial community (*e.g.*, nitrification, denitrification, cellulose decomposition, methanogenesis). Bioassays which have been used to evaluate the toxicity of wastes in the environment include the Ames *Salmonella typhimurium*/mammalian microsome assay, the Microtox™ assay, S.O.S. Chromotest™, Toxi-Chromotest™ and whole organism tests (*e.g.*, earthworm, fish, daphnia).

Of particular value with respect to environmental applications are the Microtox™ assay, S.O.S. Chromotest™, and Toxi-Chromotest™ systems. These assays are generally more rapid, less expensive and less reliant on technical expertise than the Ames test. The Microtox™ assay is an aqueous toxicity assay which measures the light output produced by a suspension of marine luminescent bacteria in response to an environmental sample (McFarland *et al.*, 1991). Bioluminescence of the test microorganism depends on a complex chain of biochemical reactions, which when inhibited (by a pollutant) results in a measurable reduction in bacterial luminescence. The S.O.S. Chromotest™, and Toxi-Chromotest™ systems are based on similar principles but possess the added simplicity of direct visual detection and comparative evaluation of genotoxicity. Multiple samples (72 samples) can be simultaneously processed and examined using these systems and results can be obtained in field environments to quickly and inexpensively screen for toxicity. A number of methods have been developed, using these assays, to determine waste application loading for soil-based treatment systems, and land treatability of hazardous organic wastes (Matthews and Hastings, 1987; Symons and Sims, 1988).

Adaptation of microorganisms to a contaminated environment should result in a detectable decrease of toxicity over the course of bioremediation. Monitoring activities should thus be designed to detect these decreases in toxicity, possibly through routine rapid screening of samples with bacterial toxicity assays.

Metal ions, notably heavy metals, are often present in contaminated soils and pose several toxicological concerns. Firstly, low concentrations of several metals are essential for microbial growth. Elevated concentrations of these metals may, however, inhibit microbial growth. Non-essential metals (*e.g.*, silver, cadmium) are of concern as their concentration may inhibit

microbial growth and/or may limit the uptake of essential metals through competition for enzyme sites.

It is difficult to provide a list of general ranges of metal concentrations which are toxic to microorganisms as microbial tolerances to each metal vary widely. Instead, Table 3.8 lists specific concentrations of metals that can be tolerated by specific associated microorganisms. Above these concentrations, metals may rapidly accumulate in cells, bind to proteins, DNA and RNA and cause intracellular ion leakage (*i.e.*, potassium) and cell death.

Certain metals such as chromium are mutagenic to bacterial strains and cause cell disfunction and death (Trevors, 1991a). Several metals such as selenium and arsenic can be volatilized from soils while mercury, lead and tin can be chemically and/or biologically methylated. The volatile or methylated forms are often highly toxic and thus these activities may result in increases of toxicity. Mercuric chloride can be transformed to elemental mercury (Hg^0) by the mercuric reductase enzyme found in selected bacterial strains. The elemental mercury produced is however highly volatile and extremely toxic.

Characterization of the site geochemistry (3.2.1) should include an initial scan (*i.e.*, ICP spectroscopy) of the concentrations of metals, both essential and inhibitory, in the contaminated matrix to identify concentrations of metals which may inhibit successful microbial activity and hence bioremediation. The potential for several of the aforementioned metals to be converted to more toxic forms should be assessed and the potential influence on microbial activity evaluated.

Bioremediation of contaminated environments containing metal concentrations which are toxic or inhibitory to microorganisms may require treatment by metal removal or immobilization. Metals in contaminated soils may be removed by solvent extraction or geochemical treatment (*i.e.*, metal precipitation by oxidation). Metals in water may be removed by appropriate chelating/sorbing treatments (*i.e.*, columns or immobilized cells which accumulate and remove the metals), or by use of membrane filtration or reverse osmosis procedures.

3.1.3 Contaminant Distribution and Mass

The distribution of contamination will be important for:

- identifying source areas (and areas of high concentration) which may inhibit microbial activity and which may require remediation using an alternate technology;
- selecting the bioremediation application; and
- estimating the total contaminant mass which influences the duration and cost of cleanup.

3.1.3.1 Source Characterization

Locating contaminant sources and environmental 'hot spots' will be largely dependent on the physical nature of the contaminant(s). Dissolved contaminants will migrate with groundwater and may become sorbed to solid matrices. Dissolved phase sources, will be relatively easier to delineate and treat using current treatment technologies. Residuals will be more difficult to locate and treat and if not detected during field investigations, residuals may re-contaminate both saturated and/or unsaturated zones.

Sources and 'hot spots' in the form of non-aqueous phase liquids (NAPL) are difficult to detect and remove from the subsurface. NAPLs are problematic because they are essentially immiscible with water and migrate through the subsurface as a separate phase (Rittman *et al.*, 1991). As these contaminants migrate through the subsurface, they can become trapped as residual lenses in the unsaturated and saturated zone and as pools at or below the watertable (Rittman *et al.*, 1991). Trapped NAPLs serve as a long term source of contamination, slowly dissolving into the aqueous phase.

The behaviour of NAPLs is categorized based on their density. LNAPLs (*i.e.*, petroleum products) are less dense than water and as such rest on top of the watertable. LNAPLs can be effectively removed by skimmer and extraction technologies. DNAPLs (*i.e.*, aliphatic chlorinated hydrocarbons) are more dense than water and so sink to the bottom of the aquifers, often resting on aquitards or becoming trapped in fractures. Locating and removing DNAPLs is often unfeasible and in some cases, impossible. Table 3.5 provides the densities of a number of organic contaminants. Contaminants with densities greater than 1.0 and low solubilities may exist in the subsurface as non-aqueous, free-product phase.

Residuals and free-phase contaminants may present toxicity problems owing to their elevated concentrations. This will result in zones of microbial inactivity, which may require treatment with an alternative technology.

Detection and characterization of sources, residuals and 'hot spots' will be key in determining the feasibility of all bioremediation applications (on-site and *in situ*). If the sources cannot be reliably located and delineated (including NAPLs and residuals), success of any on-site or *in situ* bioremediation effort cannot be ensured. Source toxicity (section 3.1.2.5) must also be identified and its effect on *in situ* and on-site applications determined. For *in situ* applications, alternative treatment methods may be required for source removal. For on-site applications, it may be possible to dilute elevated source concentrations to reduce toxicity.

Comprehensive source characterization will determine the physical states associated with each contaminant requiring bioremediation. Not all contaminants will require monitoring in each phase and as such, a chemical/phase specific monitoring strategy can be designed for evaluation of treatment effectiveness (Sims, 1990).

If remediation objectives require very complete destruction of small masses of contaminants within isolated pockets of a site, the probability of bioremediation success will be very dependant on the heterogeneity of the formation(s) and other hydrogeological conditions (section 3.2).

3.1.3.2 Estimating Contaminant Mass

Proof of bioremediation can be obtained directly through use of mass balances provided that abiotic and biotic mass transfers can be distinguished (Madsen, 1991). It would thus seem reasonable that all monitoring programs include mass balancing to validate bioremediation. However, many factors influence the accurate determination of contaminant mass and its loss. Figure 3.3 shows the difficulty in estimating the abiotic and biotic mass losses during biodegradation in laboratory trials and in controlled-release field plots. While abiotic mass loss can be controlled and quantified in the laboratory, the level of mass loss due to uncertain processes in controlled field experiments is significant.

Generally, contaminant mass that exists only as a dissolved or vapour phase can be more accurately estimated (within half an order of magnitude). Contaminants present in soils and groundwaters as free-phase or residual liquids will increase the uncertainty of estimating the mass

(1 - 2 orders of magnitude). The degree of monitoring will also influence the accuracy of the estimate.

Initial mass estimates will be required and may be obtained from existing records or company logs. In some cases, it may be possible to calculate the mass of contaminant released by delineating the concentration distribution of the contaminant(s) within the impacted area. A spill scenario is the only case where the mass of the contaminants can be accurately known. Unfortunately, accidental release scenarios often preclude use of mass balances for confirmation since control treatments distinguishing biotic and abiotic losses will not exist. As such the use of indirect evidence for validating bioremediation will be more common.

3.2 Physicochemical Characterization

3.2.1 Geochemistry

The presence and success of microorganisms and thus the success of bioremediation depends on nutrient requirements, microbial tolerances and on critical physical/chemical factors which affect activity and growth. The interactive nature of these environmental factors complicates the task of precisely defining the controlling or limiting factors in natural ecosystems (Atlas and Bartha, 1987). Nevertheless, a single condition can often be identified that, by exceeding the tolerance limit, results in exclusion of microorganisms from a given environment (Atlas and Bartha, 1987). Physicochemical parameter determinations tend to provide average conditions prevailing in large samples and fail to consider the existence of microhabitats which can support diametrically opposed biological processes. For example, strict anaerobic bacteria (which cannot survive in the presence of oxygen) will be present in highly aerobic soils. Their survival is dependent on the presence of anaerobic microhabitats.

3.2.1.1 pH

It is known that the pH of an environment affects microorganisms and microbial enzymes directly and influences the dissociation of many molecules that influence microorganisms. Most microorganisms prefer near neutral or slightly alkaline pH and generally cannot tolerate pH extremes (Stolp, 1988). The stability of internal pH is essential for enzyme function and for generation of energy via concentration gradients across membranes. The general pH tolerance range for microbial activity is 5.5 to 8.5 (Table 3.9).

The solubility of CO_2 and other dissolved gases and the availability of nutrients such as ammonium and phosphate (which limit growth rates in many ecosystems) are governed by pH. At high pH, divalent cations such as Mg^{2+} and Ca^{2+} precipitate as carbonate limiting their availability. Also under alkaline conditions, free ammonia accumulates from conversion of NH_4^+ and adversely influences microbial growth (Stolp, 1988).

The mobility and speciation of toxic heavy metals and organic molecules are also governed by pH (Atlas and Bartha, 1987). Acidity results in an increase in the solubility of metal ions resulting in increased inhibition of metabolic systems as metal ions bind to and inactivate key enzymes.

Effective delivery of nutrients and electron acceptors will rely on a uniform distribution of pH which does not adversely affect nutrient speciation and mobility. Since organic acids are typical intermediates of mineralization processes, pH should be monitored throughout bioremediation. In some cases, it may be necessary to control pH via chemical additions, especially if buffering capacities of the material being treated are low (Rozich and Zitrides, unpubl).

pH can also affect the binding of microorganisms to solid surfaces. Surfaces of bacterial cells have pH-dependent charges while soils carry electrostatic (pH-independent) charges which are predominantly negative. As a result, bacterial cells and soil generally repel each other and reduce attachment of the cell to the soil surface. Changing the pH may reduce or increase repulsion and may determine if allochthonous microorganisms will be transported and distributed from the point of inoculation, or if they will simply aggregate at the inoculation point.

3.2.1.2 Eh/redox

Many enzymatic reactions are oxidation-reduction reactions in which one compound is oxidized and another compound is reduced. The ability of an organism to carry out oxidation-reduction reactions depends on the redox state of the environment. In groundwater systems or solutions, the proportion of oxidized to reduced components constitutes the redox potential (Eh) (Atlas and Bartha, 1987).

The redox potential is greatly influenced by the presence or absence of molecular oxygen. Certain reactions, such as sulphate reduction or methanogenesis only occur under negative Eh values (Table 3.9). Kobayashi and Rittman (1982) determined that significant reductive

dechlorination, a process key to bioremediation of chlorinated solvents, only occurred in environments where the redox potential was below 0.35V. The dominant microorganisms (obligate aerobes, obligate anaerobes or facultative microorganisms) and thus the dominant biodegradative pathways will be influenced by the redox environment. Essential nutrient elements such as iron and manganese are also influenced by the redox environment as they are soluble at low redox potentials but precipitate in oxidizing environments (Atlas and Bartha, 1987).

It is difficult to measure Eh in natural habitats and Eh heterogeneity is great. As such, obtaining representative samples for treatability studies is difficult. Care must be taken in sampling procedures to retain redox conditions so that treatability studies will provide representative evidence of reactions that will occur in the sampled environment. Table 3.9 provides the redox potentials at which several microorganisms function and at which certain microbial processes operate. Generally, activity of aerobes and facultative aerobes requires Eh values exceeding 50 mV while anaerobic activity requires that the Eh is below 50 mV.

The redox state of an environment will dictate which metabolic pathways will predominate in the biodegradation of contaminants. This may allow *a priori* identification and evaluation of the metabolites, toxicities and biodegradation rates which are likely to be involved. Treatability studies (*i.e.*, microcosms, section 4.0) must ensure that the redox state is carefully retained and maintained so that treatability study results provide an accurate assessment of microbial capabilities. Many bioremediation strategies intentionally alter the redox environment (*i.e.*, addition of oxygen) to establish and/or maintain desired redox conditions. In these cases, treatability studies should focus on creating and maintaining the desired manipulated environment.

Bioactivity often results in the depletion of nutrients and electron acceptors (*i.e.*, oxygen, nitrate, sulphate) which influence or control the redox state of a contaminated environment. Eh differences between contaminated and uncontaminated zones owing to bioactivity can be used to monitor microbial activity and provide evidence for bioremediation.

3.2.1.3 Temperature

All microorganisms have characteristic temperature ranges and optimums for growth and reproduction (Atlas and Bartha, 1987). In general, higher temperatures that do not kill microorganisms result in higher metabolic activities (*i.e.*, increased oxygen consumption). In

most bioremediation activities, the optimum temperature will be between 20°C and 30°C. However, there can be significant microbial activities at temperatures outside this range. Table 3.9 provides ranges of temperature and optimums for various physiological groups of microorganisms. Temperature will also influence chemical reaction rates (section 3.1.1.5) and abiotic processes such as solubility (section 3.1.1.2) and volatilization (section 3.1.1.3).

Evaluation of bioremediation strategies must consider the temperature at which substrate metabolism will occur. The rate of biodegradation and thus project duration will be dependant on the temperature. Winter bioremediation activities in northern latitudes, notably on-site applications, may require provision of physical containment facilities (*i.e.*, greenhouses) for control of temperature (Rozich and Zitrides, unpubl).

Treatability studies (*i.e.*, microcosms, section 4.0) must attempt to simulate the temperature at which field level applications will occur. Results of laboratory simulations conducted at room temperature will have no relevance to rates and kinetics likely to occur in the field. Manipulation of temperature to enhance bioremediation should also be addressed where possible. Monitoring activities should include quantification of seasonal fluctuations to ensure continual microbial activity. This will be particularly important for above and shallow ground (*e.g.*, landfarming) treatment strategies.

3.2.1.4 Osmotic pressure

Microorganisms must cope with osmotic pressure resulting from differences in solute concentration on opposite sides of a semipermeable membrane (Atlas and Bartha, 1987). Microorganisms possess certain adaptive capabilities that allow them to tolerate a range of osmotic pressure. However, few bacteria (halophiles) can tolerate the osmotic pressure of saline brines, salt marshes and seawater environments. High salt concentrations tend to denature proteins thus reducing enzymatic activity.

Generally, reduction in biodegradation activity because of osmotic pressure is not a concern in bioremediation. However, microbial activity such as reductive dechlorination may result in release of sufficient quantities of chloride ions to impact upon the osmotic pressure. The presence of chloride ions is easily detectable using a chloride ion specific electrode/meter and is good evidence that biologically mediated dechlorination is occurring (section 3.1.2.1). Trevors (pers. comm) and Major *et al.* (1991) have used this technique to successfully measure the

release of chloride ions during the biodegradation of pentachlorophenol (PCP) and tetrachloroethene (PCE), respectively. Monitoring of changes in osmotic pressure will be of particular concern in on-site treatments which are unable to feasibly maintain osmotic levels via dilution.

Solute concentration may also have a marked effect on the transport of allochthonous microorganisms. Gannon *et al.* (1991) have shown that the transport of a *Pseudomonas* strain in a saturated laboratory sand column is enhanced when the carrying solution is switched from 0.01 M NaCl to distilled water. They postulate that the change in sorption of the microorganisms was due to changes in the cation-exchange capacity of the soil (see section 3.2.2.5).

Lastly, dissolved salts in the aqueous phase may either increase or decrease the solubility of a contaminant by processes referred to as "salting-in" or "salting-out". Bohon and Clausen (1951) found that silver nitrate substantially increases the solubility of toluene (salting-in), whereas potassium nitrate reduces the solubility of toluene (salting-out). Both sodium chloride solutions and seawater have also been shown to reduce the solubilities of toluene and benzene (Rossi and Thomas, 1981; Price, 1976; Sutton and Calder, 1975). Price (1976) examined the solubilities of benzene and toluene over a range of sodium chloride concentrations and found the solubilities of both decreased significantly with increasing salt concentrations. Thus, the presence of dissolved salts may play a role in the solubility of contaminants and thus may influence contaminant availability and toxicity.

3.2.1.5 Moisture content

The moisture content, or water activity, provides quantification of water available for microbial use. It depends on the number of moles of water and solute and on the activity coefficients for water and the solute (Atlas and Bartha, 1987). Water activity can be reduced by the presence of solutes (osmotic forces) and by adsorption to solid surfaces (matric forces). Moisture content can be determined in the field by measuring the bulk density of the solid matrix. Generally, microbial activities are associated with moisture content values of 60 to 80% of the water holding capacity (WHC) (Table 3.9).

Microbial activities in soil generally fluctuate with the moisture content; the lower the water content, the lower the activity. The moisture content of the soil and its distribution must be determined and monitored to ensure that adequate water is available to allow bioactivity. For

biofarming, it will be important to keep windrows moist by periodic spraying with water. On-site applications may also require addition of water to replace loss through evaporation (Rozich and Zitrides, unpubl). Increases in water content should be monitored to ensure waterlogging does not result in shifts from aerobic to anaerobic metabolism. Unpredicted shifts in metabolism may produce adverse metabolites, or may alter conditions hindering the eventual re-establishment of the desired aerobic microorganisms.

Several non-microbial processes such as volatilization and diffusion of gases, and infiltration and movement of contaminants, will also be influenced by the moisture content of soils. Bioventing of gases will require sufficient water to maintain microbial activity.

3.2.1.6 Nutrients

Mineralization of organic matter provides the carbon required for growth of active microorganisms, however, organic compounds are often in limited supply in natural ecosystems. While natural carbon cycling occurs, in general, this turnover is not sufficient to meet the growth needs of a microbial community. The presence of organic contaminants in ecosystems provides a source of carbon for growth. However, if an excess of available carbon is not coupled with sufficient levels of other required nutrients (*i.e*, inorganic nutrients), unbalanced growth may result.

Inorganic nutrients are essential for the growth and maintenance of microorganisms. The chemical form of these contaminants dictates their availability and can influence their toxicity. The following inorganic nutrients are required as part of microbial metabolism and growth:

- Oxygen is an electron acceptor required in most aerobic metabolic pathways. The initial step in oxidation of many contaminants involves the incorporation of one oxygen molecule from molecular oxygen into the oxidized product (*i.e.*, incorporation of O into the aromatic ring nucleus)

- Carbon dioxide is required for both autotrophic and heterotrophic growth. It is available in solution as carbonate, bicarbonate or in gaseous form.

- Nitrogen is required for amino acid, protein, purine and pyrimidine biosynthesis. It is available to few microorganisms (N_2-fixing bacteria such as *Azotobacter* spp., *Rhizobium* spp.) in its inert gaseous form and is more commonly available as nitrate, nitrite or ammonium. Some microorganisms use nitrate or nitrite as alternative electron acceptors (or in conjunction with oxygen) in respiration pathways (denitrification).

- Phosphate is required for microbial generation of ATP and for nucleic acid (DNA, RNA) and membrane (phospholipid) synthesis.

- Sulphur is required in the formation of proteins and other molecular components.

- Iron is required in cytochromes and several iron proteins.

- Magnesium is required for ribosomal function, stabilization of microbial cell wall and as a co-factor for enzyme activity.

- Chloride ions are essential for maintenance of membrane function as is sodium and potassium. Potassium is the predominant ion inside microbial cells.

- Trace metals (*i.e.*, Mn, Cu, Zn, Mo) are required for enzyme and co-enzyme functioning

Initial characterization of the contaminated environment should include analysis for the levels of nutrients and electron acceptors present in the matrix to be treated. These nutrients will likely be rapidly depleted during periods of microbial activity, thereby limiting bioremediation. As a result, addition of nutrients and electron acceptors to groundwater and soil will often be required to maintain or initially stimulate microbial activity. Table 3.9 suggests levels of key nutrients required for bioactivity and bioremediation.

Nitrogen and/or phosphorous are typically added as inorganic salts (*i.e.*, ammonium chloride, ammonium nitrate, sodium nitrate, sodium and potassium phosphate, orthophosphate) to achieve concentrations in the groundwater of 1 to 100 ppm of nitrogen and/or phosphorous (BEAK, 1990). Iron, manganese and magnesium are added as trace nutrients (10-100 ppb) usually in the form of chloride or sulphate salts (BEAK, 1990). Since these nutrients are readily soluble in water, delivery systems are designed so that nutrients can be transported through areas of contamination, converting contaminated areas into bioactive zones (Brubaker, unpubl).

Table 3.10 lists the terminal electron acceptors and reaction products of selected microbial processes. Oxygen, when available, is the predominant electron acceptor in aerobic metabolic reactions. Many bioremediation projects rely on the controlled delivery of oxygen through engineered systems to provide an adequate supply of electron acceptors. A direct relationship between the mass of oxygen required and the mass of the contaminant that can be biodegraded exists, assuming that molecular oxygen is the only oxidant. In general, the amount of oxygen required for a microbial process is approximately three pounds of oxygen per pound of biodegradable organic present within the treatment area (Brubaker, unpubl).

Nitrate or nitrite can be also be used as an electron acceptor for denitrification in oxygen depleted (anaerobic) zones of contamination. Specific enzymes (reductases) and catabolic activities are induced under denitrifying conditions which result in biodegradation of the organic compounds. Recent studies (Brubaker, unpubl) have demonstrated that PAHs and other organic compounds can be degraded by denitrifying bacteria. The use of these alternate electron acceptors, while limited in current commercial practices, has significant potential provided hydrogeological conditions allow containment of the introduced nitrate/nitrite.

Batterman and Werner (1984) first documented the use of nitrate to clean up an oil spill in the upper Rhine catchment. Groundwater was stripped of volatiles, amended with nitrate, ammonia and ortho-phosphate and reinjected into the contaminated aquifer. Approximately 75% (16.6 tonnes) of the total hydrocarbons (coupled with consumption of 50 tonnes of nitrate) were removed from the pore volume of the sand after 300 days. A more comprehensive study of BTX biodegradation under denitrifying conditions (using appropriate field controls and mass balances) was undertaken by Berry-Spark *et al.* (1988) who demonstrated toluene and xylene (but not benzene) removal concurrent with nitrate reduction.

Nutrient requirements will vary with each bioremediation effort and must therefore be addressed for each case. Redox conditions (section 3.2.1.2) will dictate the metabolic pathways which will be used and thus will determine the electron acceptors that will be required. pH (section 3.2.1.1) will influence the speciation and availability of certain nutrients and electron acceptors and so must be defined.

The design and implementation of nutrient delivery systems, where required, must be addressed. These considerations will be significantly more important for *in situ* strategies in which the contaminated environment cannot be easily and cost-effectively manipulated. Delivery of nutrients in *in situ* bioremediation strategies must overcome clogging and precipitation of delivered nutrients. If the proper nutrients and electron acceptors cannot be uniformly distributed in sufficient quantities, the success of bioremediation cannot be ensured. Shifts in terminal electron acceptors as a result of inadequate supply or distribution may induce changes in the microbial community structure, thereby limiting success of a bioremediation strategy (White, 1988).

Detection of changes in reactants (O_2, nitrate, sulphate & reactants) and products (CO_2 & intermediary metabolites) indicative of known metabolic processes can be monitored to provide indirect evidence of bioremediation. Strength of the evidence is improved if loss of contaminant and nutrients is shown relative to loss of a conservative tracer like bromide. Ideally, stoichiometric relationship should be evident between the amount of nutrients consumed, loss of contaminants, and production of end-products and biomass. Unequivocal evidence of bioremediation is the formation of radiolabelled daughter and end-products from a radiolabelled contaminant.

3.2.2 Hydrogeological

Characterization of the hydrogeological environment will assist in predicting contaminant transport, nutrient distribution and microbial activity. Successful application of bioremediation will thus be dependant on an accurate characterization of the hydrogeological environment. The delineation of hydrogeological characteristics will be crucial for *in situ* treatments and will be of concern in the pumping portion of on-site strategies. A brief review of the characteristics of the saturated and unsaturated zones is provided and is followed by the hydrogeological information requirements.

The saturated zone occurs below the watertable and consists of the soil zone where the moisture content equals the porosity. Contamination of the saturated zone can consist of both soil contamination and/or groundwater contamination depending on the contaminant characteristics (see section 3.1.1). In general, the saturated zone can be divided into aquifers and aquitards. Aquifers can be further subdivided into unconfined (water table aquifer) or confined aquifers which are bounded by low permeability aquitards (Freeze and Cherry, 1979).

The unsaturated zone, often referred to as the vadose zone, is the region extending from the ground surface to the upper surface of the principal water-bearing formation (Sims, 1990). The vadose zone is in contact with the atmosphere through its network of pores and is also in contact with the saturated zone through the capillary fringe. Contaminants in the vadose zone may adsorb to soil particles or may volatilize and be retained within the pore spaces. Diffusion of gases within the vadose zone may redistribute contamination and may hinder accurate mass balances. Fluctuations in the watertable and/or infiltrating water may contact soil gas and introduce contamination to underlying aquifers.

In general, as the complexity of the geology increases, notably heterogeneity, so does the complexity of geochemical and microbial transport and thus the likely success of *in situ* processes decreases due to impracticality and costs required to obtain adequate control.

3.2.2.1 Delineation of the Saturated and Unsaturated Zones

The thickness and extent of the saturated and unsaturated zones and the location of confining units and discharge areas will aid in delineating contamination and in predicting the transport and distribution of the contaminant(s). The potential for further contamination of aquifers or vadose zones and the potential impact of further contamination should be addressed. For example, passive bioremediation (in which bioremediation occurs within the migrating plume) may not lower contaminant concentrations in sufficient time to prevent impacting drinking water supplies or discharges to surface waters.

The presence, distribution and size of fractures will also influence the distribution of contaminants. For example, fractures in confining units may permit the migration of contaminants into otherwise isolated aquifers. Fracturing in the aquifer, aquitards or vadose zone should be assessed to ensure containment and availability of contaminants for biodegradation.

Another characteristic which should be considered is the depth to the watertable. At sites with shallow watertables, injection of water through wells or trenches may cause flooding. Shallow injection points limit the amount of hydraulic head which can be applied to an injection system, thereby limiting the area of influence of injection wells. This will affect the rate of travel of injected water and nutrients into contaminated areas, thereby influencing treatment time, and capital costs (Brubaker, unpubl).

3.2.2.2 Hydraulic Conductivity

Hydraulic conductivity (K) quantifies the overall ability of a porous medium to conduct water. A similar K value for vapour transport can be determined for bioventing strategies. Characterization of contaminant flow and of the behaviour of nutrients and other key geochemical species during bioremediation will be dependant on the distribution of K. For example, if two units both have high K, but one unit has a K value one hundred times that of the second, then essentially all flow will occur in the first unit, regardless of the high K of the second unit. The success of any injection or extraction system (including wells, infiltration galleries and drainage ditches) for control of contaminant movement and delivery of essential electron acceptors and nutrients will thus depend on identifying the distribution of hydraulic conductivity in the contaminated matrix (Rittman *et al.*, 1991).

Many bioremediation strategies withdraw contaminated groundwater, remove contaminants, amend the water with nutrients and inject the water back to the contaminated zone. Hydraulic conductivity will control the rate at which water can be withdrawn as well as the rate that the nutrients can be delivered back into the contaminated matrix. Ideally, injection and recovery wells should be separated by a distance that allows injected, nutrient-amended water to reach areas of contamination shortly after injection, so as to limit time for nutrient speciation, precipitation, and sorption (Brubaker, unpubl).

Aquifer tests (pump and slug tests) can be used to estimate K in the field so that these systems can be appropriately situated. Measurement of the heterogeneity (spatial distribution of K) will be difficult and may influence efficiency of implemented systems.
Figure 3.4 provides the range of hydraulic conductivities associated with different consolidated and unconsolidated deposits. Current bioremediation projects generally centre on more permeable, unconsolidated deposits where diffusion of gaseous contaminants and/or movement of groundwater can be controlled. Typical hydraulic conductivities of these deposits are in the

range of 10^{-5} to 10^{-3} cm/s while pumping rates of these deposits range from 25 to 380 L/min and flow rates range from 0.6 to 800 m/yr (BEAK, 1990).

Provided that contamination is not too deep, low K soils can still be treated on-site. This initially requires amending excavated soils with bulking agents (sand, wood chips) which increase K, or mixing the surface soils with bulking agents *in situ* (*i.e.*, landfarming).

Use of bioremediation may also be feasible as a containment method for contaminants in low permeability, low K zones. While the contaminant(s) are not necessarily removed, their distribution is limited. Major *et al.* (1991) contained chlorinated solvent contamination (PCE to VC) in a low K aquifer with anaerobic activity, even though the microbial population was sparse (10^5 CFU/gdw soil) and stressed.

The moisture content of the vadose zone is generally not sufficient to allow steady state flow. As such, moisture content and not hydraulic conductivity will be the more important measurement parameter (section 3.2.1.5). In the vadose zone, distribution of contaminants will be a function of contaminant volatilization (section 3.1.1.3) and of the matrix permeability (section 3.2.2.4).

3.2.2.3 Hydraulic Gradient

Direction and magnitude of the hydraulic gradient control the movement of groundwater and associated contaminants and will be key in determining contaminant distribution. Hydraulic gradients help in identifying recharge and discharge zones and will help in proper placement of recovery and infiltration systems. *In situ* bioremediation will likely alter or may require alteration of hydraulic gradients and so these should be evaluated and monitored.

Simple observation piezometers are typically used to determine the hydraulic gradient. Multi-level piezometers or piezometer nests are perhaps more useful as they provide estimates of both vertical and horizontal components of groundwater flow (Rittman *et al.*, 1991).

3.2.2.4 Porosity and Permeability

The porosity of a geologic deposit is defined as the percentage of void space that materials contain (Freeze and Cherry, 1979). In effect, the porosity will determine the amount of liquid

(*i.e.*, water and/or contaminant) that may be retained in a geologic unit. Porosity will vary with and within the geologic deposits that make up the contaminated system(s).

The porosity of the saturated zone will be important as it will define the mass of contaminant that may be retained and may be in contact with groundwater. The porosity will also affect the hydraulic conductivity of an aquifer. In general, the higher the porosity, the higher the hydraulic conductivity. In the vadose zone, porosity will influence the amount of residual contamination which may be held in the pores of a solid matrix and will determine the mass of gaseous contaminant that may be retained within the unit(s).

Groundwater extraction may result in the dewatering of clay layers which may lead to changes in porosity and compaction of geologic deposits. Alternatively, the addition of nutrients may cause clogging and decreased permeability as a result of precipitation of the nutrients or as a result of microbial growth. Changes in porosity/permeability may alter flow conditions thereby influencing the success of bioremediation.

Permeability is defined as the ability of a geologic material to allow passage of liquid. Although permeability and porosity are often related, a porous material is not always permeable. For example, clay is porous but does not allow water to pass through.

The permeability of geologic deposits will be key to the migration of contaminants (dissolved or free phase) as both liquids in the saturated zone and as gases in the vadose zone. Permeability will be responsible for the heterogeneity of a site as zones of low permeability (*i.e.*, clay lenses) will control groundwater and gas movement. The average hydraulic conductivity will be influenced by the permeability of the materials involved. Figure 3.4 provides the range of permeabilities associated with various geologic deposits.

The average permeability of site materials and lenses of low permeability should be identified, prior to *in situ* bioremediation. The distribution of nutrients and electron acceptors to areas of low permeability may be limited, resulting in low microbial activity and the presence of contaminated zones which are not treatable by bioremediation. On-site treatments may overcome problems of heterogeneity and limitations of permeability by dilution and/or by mixing of low permeability materials with those of higher permeability.

Permeability will also be a consideration during the addition of allochthonous microorganisms because it may prevent their transport, limiting their distribution.

3.2.2.5 Cation Exchange and Particle Size

Cation exchange is a property commonly associated with clay minerals, whereby cations are exchanged between the surfaces of minerals and their surroundings. The transport of contaminants, nutrients or added microorganisms through clay minerals or lenses with high cation exchange properties may be limited by binding of these components to charged mineral surfaces. Limiting the mobility and availability of added nutrients or microorganisms may decrease the success of a bioremediation strategy. Table 3.11 provides ranges of cation exchange capacity for several clay minerals and organic matter.

Many cations involved in ion exchange (*i.e.*, calcium, magnesium, manganese, iron) are important solutes of the cytoplasmic environment required for the activity of numerous enzymes. Certain cations, notably calcium, are also required in the stabilization of structural components of the bacterial cell wall (Atlas and Bartha, 1987). Decreased availability of these divalent cations as a result of their exchange with clay minerals may result in deficiencies which limit microbial activity and growth. In addition, toxicity of metal ions exchanged and bound to the surface of clay minerals may inhibit bioactivity.

Ammonia can be lost through ion exchange with other cations in clays and soils and phosphate can interact with exchanged cations, notably calcium and magnesium. While these processes do not make the nutrients unavailable, they do limit their transport and may affect their formulation. The association of such nutrients with clays may also lead to dispersion or swelling of clay particles (Brubaker, unpubl). The swelling of clays can result in reduced permeability (section 3.2.2.4) and hydraulic conductivity (section 3.2.2.2).

Bacterial surfaces possess a negative charge which, like the cation exchange of soils, can be affected by solutes and pH changes (sections 3.2.1.4 and 3.2.1.1). Cations bound to the surface of a clay possessing high cation exchange capacity may attract and bind negatively charged bacteria and as such, may limit the distribution of added microorganisms.

Cation exchange is significantly influenced by the pH of the soil environment. In general, as the pH of a soil increases, so does its cation exchange capacity. Table 3.12 shows the effect of pH on cation exchange.

Anion exchange, a property similar to cation exchange, is of less concern as most mineral surfaces possess negative charges and thus repel anions. Attraction of anions to a positively-charged mineral surface (*e.g.*, clay edges, hydrous oxides, allophane) is often referred to as nonspecific anion reaction. Of more concern is the attraction of anions (*e.g.*, phosphate, nitrate) to the cations bound to mineral surfaces. Together, non-specific reaction and attraction to cations bound to mineral surfaces may serve to limit effective distribution of anionic nutrients within a contaminated matrix.

A general estimate of the particle size distribution at various spatial locations should be obtained as particle size will determine the space and particle surface area available for colonization by microorganisms (Rittman *et al.*, 1991). The particle size will also influence the permeability and hydraulic conductivity of the solid matrix and will dictate the ability to effectively distribute nutrients within the matrix. Particle size determination will be notably important in clays and silts where adequate surfaces or pore spaces may not be available to retain moisture or provide appropriate microbial habitats. Table 3.11 provides ranges of surface area associated with several clay minerals and organic matter. In general, the larger surface areas should better support microbial growth.

To date, little information and few examples are available to define the effect of a given particle size on hydrogeological conditions and on microbial activity, however, the role of particle size in successful bioremediation has been addressed by several researchers (Rittman *et al.*, 1991; Sims, 1990).

3.2.2.6 Natural Organic Carbon

The natural organic carbon of soil and groundwater can be measured by several conventional means, notably Total Organic Carbon (TOC), fraction of organic carbon (foc) or dissolved organic carbon (DOC). The DOC is a measure of the organic carbon dissolved in filtered groundwater samples. The foc measures the carbon adsorbed to soil surfaces and generally does not include the dissolved organic carbon. The TOC provides a measurement of the total organic carbon both dissolved and adsorbed to soil. While the results of each measurement are not equal,

it is strongly suggested that at least one of the measurements be made to assess the possible influence of natural organic matter on bioremediation.

The methods for measuring TOC and foc are similar and involve trapping and removing the water and inorganic carbon (*e.g.*, carbonates, CO_2) from a sample. The sample is then rapidly and completely combusted in a quartz tube furnace set at 800°C and the CO_2 produced is detected using a non-dispersive infrared analyzer, selective for CO_2. The expected range of the system is 0.01 to 20% but the system can detect as low as 0.007% with reasonable accuracy (Churcher and Dickhout, 1987). For a more complete description of the methods of TOC/foc analysis, the reader is referred to Churcher and Dickhout (1987).

The natural organic content of an aquifer should be determined as it may influence contaminant sorption (section 3.1.1.4) and mass balancing during monitoring (section 3.1.3.2). Contaminants and added nutrients may sorb to aquifer materials, notably humic and fulvic acids, and thus their availability may be limited. Similarly, allochthonous microorganisms may sorb to organic carbon in an aquifer and thus the distribution of the desired microorganisms may be limited. Together, sorption to natural organic carbon, may limit the success of bioremediation.

Monitoring of bioremediation using the mass balance approach may be hampered by the presence of natural organic carbon. Firstly, natural labile organic compounds may serve as alternate substrates whose degradation products (*e.g.*, CO_2) are the same as those of the contaminant(s). The accumulation and detection of these common metabolic products may lead to overestimation of contaminant degradation. In addition, contaminant mass sorbed to natural organic carbon may not be detected and thus observed mass loss may again be greatly overestimated.

The presence of natural labile organics in a contaminated system may be beneficial to bioremediation as they may provide a carbon source on which microorganisms can grow. However, in general, the natural organic content is insufficient to support the microbial activity required for bioremediation. In addition, many natural labile organics cannot be used as microbial substrates. Thus, the TOC/foc of a contaminated environment does not necessarily correlate with the potential for bioremediation success and should not be used to infer probability of bioremediation success.

3.3 Microbial Characterization

Bioremediation of contaminants can occur under a wide range of environmental conditions. The initial condition of the environment (*i.e.*, geochemical and physicochemical conditions) will determine what nutrients and electron acceptor(s) are available. This will in turn determine the groups of microorganisms that will be active, their level of activity, the biochemical pathways and enzymes that will be operational, and the contaminants that can be degraded.

The microbial community changes in size and composition during bioremediation and may alter the surrounding geochemistry, thereby creating conditions which favour the activities of other microorganisms. This dynamic activity underscores the need to understand the initial status of the microbial population and how it responds during bioremediation. This insight also provides clues on how to both evaluate the feasibility of bioremediation and monitor and validate the process.

The microbiological properties that should be characterized are:

- biomass size of the total microbial population, or subsets thereof;
- composition of the microbial community;
- activity and growth of the microbial population; and
- distribution of the above three properties.

These microbiological properties are described in sections 3.3.1 (biomass size), 3.3.2 (microbial population composition), 3.3.3 (microbial activity/growth), and 3.3.4 (microbial distribution).

3.3.1 Biomass size

An initial determination of the biomass size of contaminated and uncontaminated zones will be required to assess the feasibility of bioremediation. In most cases, initial biomass levels will be unpredictable and possibly low as microbial communities exhibit homeostasis. Comparing the biomass in and outside of the zone of contamination may indicate if bioremediation is feasible or is occurring. Transformation, and ultimately mineralization, of organic contaminants may be accompanied by an increase in biomass size provided bioactivity is not inhibited by contaminant toxicity or by the physical state of the contaminant(s). The biomass size of contaminated zones should increase in response to contaminant metabolism while the biomass size in uncontaminated

zones should remain static (except for fluctuations within a range inducible by normal environmental factors such as moisture content and temperature). Comparison of the biomass size in both contaminated and uncontaminated zones should thus provide evidence of microbial adaptation and activity.

As with all assessments, comparative biomass evaluation must be based on a statistically valid number of samples from within and outside the zone of contamination. It is possible that samples taken from uncontaminated (pristine) areas have been impacted by undetected by-products (those not analyzed for) generated in the contaminated zone. Uncontaminated soil and groundwater samples should be taken "upgradient" from the zone of contamination to ensure their integrity. If this simple precaution is not taken, it may be possible to conclude that there is no significant differences between biomass measurements in the contaminated and uncontaminated zones, and that the indigenous (or allochthonous) biomass is not responding to the contaminants (*i.e.*, a false negative).

Treatability studies should attempt to stimulate microbial activity through addition of nutrients or modification of environment conditions. If no biomass is present or if adequate response cannot be obtained with initial biomass levels, the addition of allochthonous microorganisms may be considered for bioremediation of the contaminants.

Monitoring activities should include periodic determinations of biomass size over the course of bioremediation. Comparison of *post priori* biomass against initial biomass values should provide indication that microorganisms are stimulated by the presence of the contaminant(s). Inhibition of bioactivity by high contaminant concentrations may be reflected by such biomass monitoring. Bioremediation can also be assessed during monitoring by comparing the total biomass to subgroup biomass (*i.e.*, genus or functional physiological group biomass). Adaptation of specific degrader-microorganisms and successful bioremediation should result in selection of specific groups of microorganisms in zones or contamination. Table 3.13 presents and interprets four biomass response scenarios which may be observed during bioremediation.

Because of the importance of the total microbial biomass in evaluating and monitoring bioremediation, it is critical to have analytical techniques that provide reasonable estimates of the biomass. Isolation and subsequent culturing of microorganisms using classical microbiological methods (*i.e.*, petri plates) often lead to gross underestimation of the numbers of microorganisms, while direct counts are overestimated by inclusion of non-viable bacteria. Classical culturing

methods (spread and pour plate, most probable number techniques) are limited because they rely on recovering soil and/or groundwater microorganisms which are viable and which can grow on the media selected for their cultivation. Typically, only 1 to 10% of the total biomass can be enumerated by culturing techniques.

Alternative methods for determining microbial biomass include estimation of muramic acid content, estimation of ATP levels, enzyme activity assays, and phospholipid fatty acid (PLFA) analysis (White, 1988). Each method has its associated benefits and limitations, which will not be discussed herein. Of these methods, lipid analysis provides the most representative measure of viable cellular biomass. Membrane phospholipids are found in all cellular membranes (but not in storage lipids), have a rapid turnover in living cells, are rapidly hydrolysed on cell death, and are found in constant amounts in bacterial cells in nature (White, 1988). Under conditions expected in natural communities, bacteria contain relatively constant proportions of their biomass as phospholipids. Analysis of the phospholipid content in soils thus gives a measure of the viable cellular biomass when compared to measurement of other cellular components (*i.e.*, enzymes, ATP, muramic acid) (White, 1988).

3.3.2 Microbial Community Composition

Initial determination of the microbial composition both in contaminated and uncontaminated zones will indicate if the appropriate degrading microorganisms are present. The effective biodegradation of contaminants often relies on interactions between different physiological groups of microorganisms and may rely on cometabolic interactions between different groups of microorgansisms. If interactions amongst groups of microorganisms are required for acceptable biodegradation of the contaminant(s), then the key species of microorganisms involved should be present in or added to the matrix to be remediated. For example, Shelton and Tiedje (1984) have demonstrated the relationship between a dechlorinating sulphidogen (*Desulfomonile tiedjie*), benzoate fermenters, methanogens and other sulfidogens. The researchers demonstrated that *D. tiedjie* could not dechlorinate 3-chlorobenzoate without the presence of the other bacteria. Thus, it would not be sufficient to add *D. tiedjie* to an environment contaminated by 3-chlorobenzoate if the additional aforementioned microorganisms were not present or added.

In the cases that appropriate degraders are not present, additions may be required. These additions should be followed with *post priori* community characterizations to ensure that the added microorganisms have survived and undertaken the task they were added for. In some

cases, several additions of the microbial strain(s) will be necessary to ensure required activity levels. Most bioremediation strategies, especially those involving the addition of allochthonous microorganisms, do not undertake microbial characterization either *a priori* or *post priori*. It is thus difficult to prove if bioremediation was achieved by the microorganisms that were paid for or by known or unknown indigenous microorganisms. Section 2.4 addresses several of the limitations and concerns over use of commercial inoculants.

Current methods for the identification of microbial community members are time-consuming and often lead to incomplete community assessments (section 3.3.1). Analysis of the ester-linked fatty acids of phospholipids is a promising technique because it provides a chemical fingerprint of the community structure (White, 1988). With this analysis, specific physiological subsets of the microbial community can be detected based on patterns of PLFA (*e.g.*, methanotrophs, sulphate reducing bacteria, methanogens, actinomycetes, Gram positive and negative populations, aerobic versus anaerobic populations, eukaryotes such as protozoans). Because this technique gives a broad picture of the microbial community it can be used to monitor shifts in community structure. Often the structure of the microbial community adapts to contamination. Community adaptation can be quantitatively assayed with this technique making PLFA analysis a valuable monitoring tool. However, this technique generally cannot be used to detect specific genus and species of microorganisms. If this information is required, then classical culturing and identification techniques should be used. Alternatively, specific DNA probes or monoclonal antibodies may be used to detect and estimate the biomass of individual microbial species.

The presence of microbial predators (protozoa) may provide an indirect measure of biomass as their numbers in zones of biodegradation are proportional to the size of biomass actively degrading the contaminants (Madsen *et al.*, 1991a). Soil protozoans prey upon bacterial cells and may reduce bacterial population density. The presence of an active soil protozoan population may result in reduction of an introduced bacterial population in a period of a few days to weeks, thus preventing success of bioremediation. The presence and success of protozoa will be dependant on the tolerance of the predators to the toxicity of the contaminant(s).

3.3.3 Microbial Activity/Growth

Biomass and community analysis provide no indication of activity and/or stress. Often it is possible to obtain plate counts from highly contaminated areas and therefore incorrectly conclude that the biomass is active in the contaminated zone. Table 3.14 provides an example of how

such assumptions can be incorrect. Based on the total PLFA measurement, a biomass of 10^5 cfu/g sediment is detectable at a depth of approximately 43m (below ground surface). However, corresponding measurements reveal no microbial activity. In this case, assumption of microbial activity based solely on the level of biomass detected would be incorrect and could potentially lead to failure of a bioremediation strategy. This example clearly illustrates the importance of activity measurements in evaluating the potential for bioremediation.

There are four parameters to consider when examining microbial activity;

- growth;
- nutritional status;
- stress; and
- metabolic activity and capabilities.

Microbial growth is generally associated with an increase in cell numbers, however, growth can also be an increase in cell mass without cell division. The use of radiolabelled uptake experiments can provide periodic growth measurements, termed snapshots, from on-site and *in situ* bioremediation treatments. Radiolabelled acetate incorporation into cell lipids provides snapshots of cell mass increase while radiolabelled thymidine uptake into DNA can provide snapshots of cell division. Together, these measurements are useful in determining if the indigenous or allochthonous microorganisms are active in the contaminated environment (Table 3.14) and if they are responding to stimulation for bioremediation.

In general, growth determinations are complicated by the fact that only a fraction of the microbial community is usually active at one time. The most direct method of determining the proportion of active cells is by autoradiography and electron or epifluorescence microscopy, however, these methods require metabolic activity in the presence of substrates in the field of view of the microscope. Problems then arise with the possibility of induction of artificially high levels of activity from the addition of the substrates.

Monitoring activities must be designed to determine increases and fluctuations in activity corresponding to utilization and depletion of the contaminant(s). Unfortunately, sediments are expensive to recover and groundwater contains only the losing bacteria, those which were unable to attach to sediments. Procedures need to be developed and refined to properly evaluate biomass and potential activity of microorganisms in groundwater (Phelps, pers comm). Potential methods

include lipid analysis, isotope studies and use of gene probes such as lux genes held in screen zones within perfusion chambers.

PLFA can also be used to assess the nutritional status of microbial communities. This is achieved by assaying the proportions of specific endogenous storage compounds relative to the cellular biomass (White, 1988). Under conditions of chemical (*i.e.*, contamination) and mechanical stress, some microorganisms will produce the polysaccharide glycocalyx. Other microorganisms produce poly beta-hydroxyalkanoate (PHA) under conditions of unbalanced growth (White, 1988). PFLA analysis can be combined with radiolabelled tracer studies to determine which subsets of the microbial community are actually growing and dividing, and which are stressed.

Adaptation or acclimation is a concept that refers to the amount of time that is required for microorganisms to adapt to certain environmental conditions. Adaptation may involve development of tolerance to certain conditions or may involve induction of enzymes or metabolic factors required for survival and activity in that environment. Observation of this type of phenomenon in response to a bioremediation effort can be taken as presumptive evidence that microbial action is operative (Rittman *et al.*, 1991). Adaptation/ acclimation response may not be evident at old contaminated sites where the microbial population has already adapted.

Changes in microbial activity can also be deduced by observing adaptation/acclimation response curves (section 3.1.2.4) with the introduction of contaminants or allochthonous microorganisms. This evidence will be coupled to subsequent increases in microbial numbers able to utilize substrate(s) in the contaminated area.

3.3.4 Microbial Distribution

The more homogenous the distribution of the requisite microorganisms, the faster and more uniform that bioremediation will proceed. Uniform distribution of biomass and of the appropriate groups of biodegrading microorganisms within the zone(s) of contamination will ensure that contaminated regions can potentially be bioremediated. The heterogeneity of the subsurface creates micro-habitats in which different (and often opposite) microbial communities exist. It will be important to determine if the variance in microbial distribution will limit successful bioremediation by indigenous microbes and if so, if treatment systems can produce more uniform

distribution of the microorganisms. Additions of allochthonous microorganisms may be required to populate contaminated zones in which biomass cannot be sufficiently stimulated.

The distribution of biodegrading microorganisms will also determine the metabolic processes most likely to occur throughout the zone of contamination. For example, source areas will become rapidly depleted of oxygen and thus reductive anaerobic bacteria may colonize the area unless an oxygen delivery system is implemented. At the boundaries of contamination, where lower contaminant concentrations (and lower oxygen demand) exist, aerobic processes may apply. To date, no bioremediation effort has combined the use of both anaerobic and aerobic biodegradation. However, since different contaminants and their metabolites are often more efficiently removed by either aerobic or anaerobic processes, the opportunity exists at multi-contaminant sites (*i.e.*, landfills) to use both aerobic and anaerobic bioremediation technologies simultaneously or in sequence for effective cleanup.

4.0 EVALUATION OF BIOREMEDIATION

4.1 Stepwise Evaluation of Bioremediation

The evaluation of bioremediation can be made by following a step-wise process which considers and evaluates the key factors influencing bioremediation, outlined in section 3.0. The basic steps for such an evaluation are identified and discussed in this section and are accompanied by a suggested flowchart (Figure 4.1) which could prove useful in evaluating bioremediation. Together, knowledge of these steps and use of the accompanying flowchart should indicate areas where additional information will be required prior to or during any course of bioremedial action. Section 3.0 provides the theoretical information and considerations with which to evaluate results of each step and highlights how the information requirements interrelate.

The seven general steps for evaluating the feasibility of bioremediation are:

1) Identification and characterization of the contaminant(s) (section 4.1.1)

2) Assessment of contaminant biodegradability through scientific literature and laboratory treatability studies (section 4.1.2)

3) Characterization of the contaminated environment including geochemical, hydrogeological and microbial characterization (section 4.1.3)

4) Bench-scale treatability studies to assess case-specific biodegradation using the proposed bioremedial strategy(section 4.1.4)

5) Assessment of potential biostimulation and feasibility of adjusting environmental conditions (section 4.1.5)

6) Pilot-scale (field) testing of bioremediation scenarios to determine time required for cleanup, level of attainable cleanup, and cost of cleanup (section 4.1.6)

7) Monitoring to validate bioremediation (section 4.1.7)

K[illegible] to each step is appropriate statistical design. This requires that an appropriate number of s[illegible]ples and replicates are used and that appropriate controls are in place.

4.1.1 Contaminant Characterization

The initial step in formulating a bioremediation strategy will be identification of the contaminant(s) of concern in the impacted environment. This step will be relatively easy in spill or known release situations (*i.e.*, leaking underground storage tanks) where records or logs are available defining the contents of the release. Identification of contaminants from landfill and other multiple release sites will be significantly more difficult and may require analysis of soil and/or groundwater samples for the full suite of contaminants.

Once the contaminants are identified the extant literature should be reviewed to determine if the contaminants have been previously biodegraded or biotransformed. As addressed in section 3.1.1, the existence of biodegradation data increases the confidence that the contaminant(s) can be bioremediated. As an additional step, the chemical structures of the contaminant(s) should be derived from available scientific literature. This step will be significantly more important if little or no information is available on the biodegradation of the contaminant(s). If there is indication that biodegradation is possible (based on the chemical structures and previous treatability studies), laboratory treatability studies should be undertaken.

4.1.2 Laboratory Treatability Studies

A critical component of any evaluation of bioremediation feasibility involves assessment of the susceptibility of the pollutant(s) to biodegradation. Laboratory treatability studies are tests which are designed to provide critical data to evaluate and ultimately implement one or more treatment technologies (McFarland *et al.*, 1991). Generally, the number and type of treatability studies will vary depending on the availability of information. The level of effort that needs to be expended on treatability studies is proportional to the uncertainty about site and contaminant characteristics: When uncertainty is low, reduced effort is needed to evaluate bioremediation, but extensive efforts are needed when uncertainty is high (Rittman *et al.*, 1991).

Laboratory treatability tests yield data that addresses the potential of a proposed bioremediation strategy to meet performance goals. Laboratory screening should provide "yes/no" determinations of the validity of a suggested biotreatment process and should quickly screen out inappropriate

strategies prior to their evaluation by costly bench- and/or pilot-scale studies. Laboratory treatability studies should also identify parameters (*i.e.*, reaction mechanisms, transport pathways) that will require specific attention during bench- and/or pilot-scale testing and should direct the focus of these subsequent studies. Laboratory treatability studies are generally not appropriate for generating design or cost data (Sims, 1990).

The techniques for laboratory screening should be as simple as possible. Screening is usually accomplished by placing small amounts (*e.g.*, 500 grams or mL) of soil and/or groundwater in contained vessels in which conditions can be modified and controlled, and biodegradation monitored. The contained environments, termed microcosms, can consist of sealed test tubes, shake flasks, serum bottles or hypovials. More complex microcosms can consist of columns or simulated reactors. Table 4.1 suggest examples of microcosm design for evaluation of several bioremediation strategies and Figure 4.2 illustrates several laboratory treatability systems.

Generally, the first step in laboratory treatability studies is to determine if the contaminant(s) can be directly degraded by alleviating simple nutrient and/or physicochemical limitations. In these cases, the disappearance of the target contaminants, compared against abiotic controls, is generally sufficient to provide an answer on the potential for biodegradation (Rittman *et al.*, 1991). Often, by providing a variety of electron acceptors, specific microorganisms or physiological function groups of microorganisms can be stimulated and survival and/or activity of the microbial population can be enhanced. Failing this, more complex biodegradation strategies (section 3.1.2.2) can be addressed, such as assessment of cometabolic or analog degradation.

Laboratory screening will be more intensive for unusual or unidentified contaminants, or those that occur in mixtures that may be inhibitory (*e.g.*, mixtures of organic compounds and/or heavy metals). Mixtures of contaminants will pose special problems in terms of the decision to use bioremediation as a treatment method. For example, mixtures of aromatics (generally biodegradable aerobically) and chlorinated aliphatics (degradable by reductive dechlorination) will provide interesting and complicated problems regarding the choice of a bioremedial strategy (aerobic versus anaerobic). Each component of a mixture will have a characteristic toxicity, recalcitrance and concentration. The choice of a bioremedial strategy will thus be dependant on the degrees of each of these characteristics and on the stringency of the regulatory requirements. Table 4.3 suggests a system which can be used in prioritizing contaminant bioremediation on the basis of toxicity, concentration, and recalcitrance of each component of a mixture. While these

these factors are suggested in focusing bioremedial activities, they should not be considered exclusive as a number of other factors (*e.g.*, regulatory requirements, hydrogeological conditions) may be significant in choosing an appropriate strategy.

The concentration thresholds (S_{min}, section 3.1.2.3) of the contaminant(s) should be determined during laboratory treatability studies, or alternatively by theoretical calculation, to ensure that the bioremedial strategy will succeed in reducing contaminant concentrations below regulatory limits. Secondary-substrate utilization strategies and/or non-steady state growth strategies may require investigation and implementation or bioremediation may require coupling with a cost-effective polishing technology (carbon adsorption).

A key goal of laboratory treatability studies should be the determination of the potential toxicity and/or recalcitrance of metabolic intermediates and end-products. The toxicity of these metabolites can be evaluated using toxicity bioassays such as those described in section 3.1.2.5.

Laboratory treatability studies should allow determination of the rates and kinetics of the chosen biodegradation process. These rates should be compared with other bioremedial strategies and alternative technologies (*i.e.*, abiotic reactions) to ensure that the chosen and tested method is appropriate.

Once laboratory screening has provided evidence in favour of a treatment strategy, bench-scale studies (section 4.1.4) and eventually pilot-scale tests (section 4.1.6) can and should be investigated. Table 4.2 defines the characteristics of the three levels of testing and includes general estimates of cost and required time for completion of each level.

A key advantage to laboratory treatability studies that should be discussed is their ability to incorporate radiolabelled substrate mineralization-mass balance approaches. Incorporation of these techniques will be of particular value for compounds whose metabolism is largely unknown. Use of radiolabel mineralization experiments will ensure that contaminants are actually metabolized and ultimately mineralized rather than transferred between phases or transformed to recalcitrant intermediates. Figure 4.3 provides a schematic of a biometry flask, commonly use in mass balance-mineralization studies using radiolabelled substrates.

For the purposes of mineralization studies, substrates (*i.e.*, contaminants) can be labelled in two ways. Biodegradation studies will likely use uniform labelling in which all carbon or hydrogen

atoms are labelled. In these experiments, it will be possible to monitor and determine the fate of all labelled mass to ensure that the substrate has been mineralized and has not just undergone an undetectable phase change (*i.e.*, volatilization). This system of mass balancing accounts for all labelled mass whether it be in soluble, extractable or volatile form, as initial substrate, intermediates or end metabolites.

Alteratively, non-uniform labelling of a growth substrate can be used in pulse-chase experiments to identify specific metabolic/biodegradative pathways. An example of non-uniform labelling consists of labelling only the methyl group of toluene to determine whether the substituent is transformed to carbon dioxide or methane. This method of labelling is generally limited to research studies and is generally not required in treatability studies assessing whether a contaminant can or cannot be biodegraded.

The most commonly labelled atoms are carbon (^{14}C), hydrogen (^{3}H), and phosphorus (^{32}P). Addition of radiolabelled substrates should address problems with artificially inducing activity by introducing a substrate for microbial growth. This will be of particular concern in studies attempting to simulate *in situ* activities of subsurface microorganisms whose activity and metabolic requirements are low. With this in mind, radiolabel substrates providing high specific activity should be used to minimize substrate additions and artificial induction of activity.

Mass balance-mineralization studies can be performed without the use of radiolabelled substrates. In general, these studies involve addition of known concentrations of growth-limiting substrate, electron acceptors and nutrients followed by monitoring of the stoichiometric disappearance of the added components and production of expected intermediates and end products such as CO_2. These changes should, if attributable to biodegradation, be accompanied by increases in biomass and changes in toxicity. Microcalorimetry can also be used to monitor heat production as a result of microbial activity, given that appropriate controls are in place.

4.1.3 Characterization of the Contaminated Environment

Laboratory treatability studies revealing contaminant biodegradability using a proposed treatment strategy should be followed by characterization of the contaminated environment. This characterization can be divided into three separate but interrelated characterizations, namely contaminant, physicochemical and microbiological characterization. These characterizations are well-defined in section 3.0 and so will only be briefly addressed.

The contaminant characterization, supplemental to the initial identification of the contaminant(s) and their chemical attributes, will involve identification of several key chemical attributes, namely solubility (section 3.1.1.2), volatility (section 3.1.1.3) and sorptive capacity (section 3.1.1.4). These attributes are both chemical and site specific and must be defined prior to treatment as each can limit the success of bioremediation. In certain cases, it will be necessary to control these attributes to prevent contaminant redistribution and toxicity.

Characterization of the contaminants will also involve delineation of the distribution, mass and concentration of the contaminants. Contaminant concentration is an important parameter since it affects toxicity, rate of biodegradation and job duration. Pretreatment of the site may be required to reduce the concentration (such as free-product recovery). Alternatively, another treatment technology which can treat high concentrations of the contaminant(s) may need to be added to the treatment chain.

The distribution and the physical nature of the contaminants will also play key roles in determining if bioremediation is a feasible treatment option. Sources existing as residuals and NAPLs may be difficult to locate and treat using biological methods, thereby precluding bioremediation as a feasible treatment technology. The waste composition variability should also be delineated since biological activity varies with waste composition resulting in inconsistent biodegradation.

Characterization of the physicochemical attributes of the contaminated environment, including the geochemistry and hydrogeological/hydrogeological conditions must also be defined prior to bench-scale studies. The pH, redox conditions, temperature, moisture content and osmotic conditions which will be encountered during full-scale bioremediation must be identified prior to bench-scale tests. Similarly, the hydraulic conductivity, hydraulic gradient, cation exchange capacity, organic carbon content and porosity and permeability must be identified. Potential problem areas should be identified and where possible, cost-effective manipulation of these conditions should be addressed.

Characterization of the geochemistry will be specifically important with regards to the activity of microorganisms and the effective distribution of nutrients, electron acceptors and microorganisms. Hydrogeological characterization will be of great importance in designing appropriate treatment systems for groundwater extraction and delivery of nutrients, electron acceptors and microorganisms.

Characterization of the microbiology of the impacted environment will involve identification of the microbial biomass, community composition, nutritional status and activity. The presence of appropriate requirements, the ability to stimulate these requirements and the ability to add microorganisms to overcome specific deficiencies should all be addressed. If treatment systems cannot effectively distribute the appropriate groups of required microorganisms, either indigenous or allochthonous, and/or if appropriate microbial activity cannot be elicited, alternative treatments may be required.

4.1.4 Bench-scale Treatability Studies

If characterization of the impacted environment reveals that the contaminants can be biodegraded in the impacted matrix, either *in situ* or on-site, and if physicochemical and/or microbiological conditions do not preclude successful bioremediation, then bench-scale testing should be undertaken. Bench-scale treatability studies are representative systems designed to verify that the technology selected can meet expected cleanup goals. These systems should generate cost and design data and should allow design of an appropriate pilot-scale (*i.e.*, field) program based on sound science and engineering (Rittamn *et al.*, 1991).

Bench-scale microcosms represent an increase in the scale of the simulated environment (*e.g.*, 100 L samples), as compared with initial laboratory biodegradability studies. Bench-scale systems can consist of columns, soil beds and/or prototype reactors which more realistically and more intensively simulate the real-world target environment (Dupont, 1991). These studies generally place an intact soil core taken from a contaminated environment in an apparatus which allows complete monitoring of soil moisture, pH, aeration, Eh, temperature, volatilization, sorption, photodegradation, leaching and biological processes. Bench-scale studies should also include assessment of fluid hydraulics and mass transfer on overall contaminant fate and transport (Dupont, 1991). Bench-scale treatability systems for evaluation of *in situ* bioventing of contaminated vadose zone, and contaminated groundwater are shown in Figures 4.4, and 4.5 respectively.

It is important that bench-scale systems simulate the bioremediation strategy as closely as possible to ensure accurate results prior to scale-up to pilot/field tests. For example, if the proposed strategy involves treatment using a slurry-phase reactor, then studies should be designed to provide data addressing retention time, solvent use, phase partitioning and sorption. *In situ*

bioremediation strategies should accurately simulate limitations imposed by hydrogeological constraints such as permeability, conductivity and geochemistry.

4.1.5 Biostimulation and Adjustment of Environmental Conditions

Biostimulation experiments should be conducted to optimize the treatment efficiency of the selected treatment during bench-scale studies. These experiments should identify the adjustments that may be required in the scale-up to field trials.

Environmental conditions may need to be adjusted to allow desired microbial activity. This may include adjustments to the pH, redox conditions, temperature and moisture content. Similarly, the hydrogeological conditions may require adjustment to improve the distribution of key elements required for bioremediation. Toxic compounds such as heavy metals may require treatment or removal. At this point bioremediation may be ruled out since it may not be possible to make the required adjustments.

4.1.6 Pilot-scale Studies

Pilot-scale testing should be designed to generate quantitative design, performance and cost data for operation of full-scale bioremediation applications (McFarland *et al.*, 1991). Pilot testing should consider field-scale limitations imposed by geochemical and/or hydrogeological constraints (*i.e.*, soil, chemical, and biological heterogeneity, permeability) and mass transfer and fluid transport limitations. Pilot tests should be performed on a representative portion of the contaminated material to simulate the proposed bioremediation scheme. The scope of the pilot test should be large enough that realistic complications will be encountered. Ultimately, pilot-scale testing, and the entire stepwise process outlined, should yield the most reliable, time and cost effective strategy for bioremediation.

4.1.7 Monitoring for Validation of Bioremediation

Section 3.0 outlines the use of each information requirement in monitoring bioremediation activities for the purpose of ultimately validating cleanup. While it has been suggested the that mass balance approach to monitoring is the most definitive proof of bioremediation, it is more likely that validation will come through logically linking several key pieces of indirect evidence.

For example, a reduction in the concentration of the target contaminant(s) below regulatory limits, coupled to increased microbial biomass (notably the appropriate physiological groups of microorganisms) in impacted zones, and decreased toxicity of the contaminant(s) to the microbial population (possibly measured using toxicity bioassays), may provide sufficient evidence that a bioremediation strategy has successfully remediated a contaminated environment. Obviously, a rigorous evaluation of monitoring program results will be required before bioremediation can be validated.

4.2 Microcosms

Microcosms are extremely useful tools in both laboratory and bench-scale treatability studies and as such deserve additional discussion. Microcosms are calibrated laboratory simulations of a portion of a natural environment in which relatively undisturbed environmental components are enclosed within definable physical and chemical boundaries for study under defined lab conditions (Pritchard, 1981). They are reliable, replicable tools for evaluating the susceptibility of a contaminant to biodegradation. Microcosms:

- are scientifically rigorous and statistically oriented surrogates for field studies;
- allow assessment of risk and prediction of transport and fate of contaminants within aquifers;
- help to evaluate the waste assimilative capacity of an environment;
- can incorporate numerous site-specific variables without requiring a detailed knowledge of what these variables actually are;
- can overcome limitations prior to scale up and optimize treatment strategy; and
- can be used to determine the predominant pathways of biotic or abiotic transformation and can more accurately determine degradation rates.

To date, there are few microcosm-field calibration studies reported in the literature. Microbial activities and biodegradation rates observed in microcosms may be different than those in the open environment. As such extrapolation of laboratory techniques and results to field trails is an uncertain process. Currently, Sturman *et al.* (pers comm) of Montana State University are undertaking a review of the existing literature to determine factors to consider for scaling-up laboratory studies to full scale bioremediation.

Because site specific processes will influence biodegradation, it is crucial that the microcosm reflects the conditions that exist at the site using the treatment strategy envisioned. This requires that authentic material be acquired (*e.g.*, subsurface soil and groundwater cannot be contaminated with non-indigenous microorganisms, and *in situ* redox conditions must be maintained during sample retrieval). The importance of this cannot be over-stressed. Any evaluation of biodegradation capabilities of the natural consortia of microorganisms will be affected by any induced changes in the nutrient, geochemical, or microbial composition and concentration. In addition, the microbial population of groundwater and soils is vastly different, and thus both must be present in the microcosm to properly assess the biodegradation of a contaminant (Thomas *et al.*, 1987).

Sampling methods may induce disturbances in ecosystem structure and function (and may lead to population shifts and enhanced microbial activity) thereby leading to erroneous treatability evaluations. It will thus be important to utilize sampling techniques which minimize the disturbance effect. Similarly, it is important that adequate quality assurance and quality control associated with the collection of representative soil and groundwater samples. Except for research sites, little attempt is made to ensure that the soil and groundwater samples are not being contaminated by surface microorganisms and drilling muds. Laboratory tests conducted with samples contaminated with surface microorganisms will lead to false conclusions on subsurface microbial activity, population composition, nutritional status and biodegradation capabilities. In addition, unrealistic testing conditions (*i.e.*, temperature, continuous shaking, aeration) can lead to extreme over-estimation of biodegradation rates and produce artificial data on what microorganisms or groups of microorganisms are involved in the degradation.

Several limitations of microcosms must be realized. Firstly, microcosms represent optimum conditions with respect to sample homogeneity and contact with solid materials, contaminants, microorganisms and nutrients. As such, laboratory studies provide information on the potential (not necessarily actual) treatment rates. In addition, microcosms have abnormally high surface

to volume ratios. Increased sorption of bacteria, nutrients or substrate as a result of increased surface area may affect (increase or decrease) activity thereby providing erroneous results in metabolism studies (Suflita, 1989b).

Currently, no single, universally approved microcosm design is consistently used by laboratories involved in bioremediation research. Microcosms can be as small as 25 mL centrifuge tubes to as large as 20 L carboys. Biometer flasks are useful for radiolabel (^{14}C) tracer studies as they are designed to trap labelled carbon dioxide ($^{14}C\text{-}CO_2$) for liquid scintillation counting. Stainless steel and PVC columns (about 10-12 cm in diameter and 50-100 cm in length) are useful for simulating vertical soil profiles. Large carboys (20 litre) are useful for aquatic microcosms. Although variability between designs is normal and not well standardized, it is more important in the evaluation of the microcosm data that there are appropriate controls and performance checks present within the design.

It is important that the hydrogeological, microbiological and contaminant parameters are quantified relative to contaminated areas prior to treatment. Field controls are lacking in many case studies (research studies are the exception). A minimum level of field control would be the comparison between treated and untreated areas within the contaminant plume. Additional controls could include a comparison between two plots to compare the effect of water addition (or circulating groundwater) with and without addition of nutrients. This type of control is thought to be necessary since disturbance effects can stimulate microbial activity or help mobilize available nutrients. A third control could consist of an uncontaminated area outside of the contaminant plume. This control would monitor fluctuations in the environment (moisture content, osmotic pressure) that could influence activities within the contaminant plume.

In-situ testing devices are currently finding favour. These devices attempt to modify conditions and monitor biodegradation without requiring the removal of contaminated matrix. In this case, disturbance of the ecosystem structure is avoided and sampling contamination is minimized. Current development of *in situ* testing devices (Gillham *et al.*, 1990) is allowing small-scale field experiments to determine the effect of various treatments on bioremediation and field controls. These devices allow abiotic control of experiments to be conducted. Future use of these testing devices will hopefully minimize the collection of non-representative data by removing the aforementioned technical problems. Figure 4.6 provides a schematic of an *in situ* column for monitoring and evaluating soil and/or groundwater contamination.

In general, if biodegradation cannot be successfully stimulated in microcosms, the chances of success in the field are minimal but if initial microcosm evaluations prove successful, the chances for success in the field are significantly improved (Suflita, 1989b).

4.3 Mathematical Models

To estimate the effects of transport and biotransformation in a bioremediation project, data from bench and pilot-scale treatability studies can be input into comprehensive mathematical models (McFarland *et al.*, 1991). Mathematical models can:

- integrate degradation, transport and partitioning data with characterization of the contaminated environment;
- simulate the behaviour of contaminants in the environment;
- predict the pathways of contaminant migration;
- identify contaminant phases requiring treatment with alternative technology; and
- approximate rates, duration and cost of the proposed biotreatment technology.

Several models have been developed for soil or groundwater applications, however, few are adapted for the specific purpose of simulating total (soil, soil-gas and groundwater) contamination. Development of such models will likely receive much attention as bioremediation continues to gain acceptance as an effective remedial technology.

5.0 BIBLIOGRAPHY

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TABLES

TABLE 2.1. List of Responding Commercial Organizations and their Involvement in Bioremediation

Organization	Apply Bioremediation	Add Allochthonous Microorganisms	Supply Allochthonous Microorganisms	Comments
Alko Biotechnology Ltd.	✔	✔	✔	
Argonne National Laboratories	✔	✔	†	
Bioscience, Inc.	✔	✔	✔	
BioTrol, Inc.	✔	✔	✔	
Biovac Environmental Services	✔	✔	✘	
CRA Advanced Technical Development	✔	✔	†	
Ebasco Environmental	✔	✔	†	
Eco-Bac, Inc.	✔	✔	✔	Distributor for Micro-Bac International.
EMCON Associates	✔	✔	†	
Enviroflow, Inc.	✔	✔	✔	
ESE Biosciences, Inc.	✔	✔	†	Formerly Hunter Bioscience, Inc.
Heritage Remediation/Engineering	✔	✔	✘	
Kiseki Inc.	✔	✔	✔	
Merkert Laboratories, Inc.	✔	✔	✔	
Polybac Corporation	✔	✔	✔	
Remediation Technologies, Inc. (ReTeC)	✔	✔	†	
Roberson Environmental Services Ltd.	✔	✔	✔	Canadian distributor for Merkert Labs.
Shell Canada Products Ltd.	✔	✔	✘	
Sussex Environmental Services, Inc.	✔	✔	✘	
Waste Stream Technology, Inc.	✔	✔	✔	
ADS Associes Ltee	✔	✘	✘	
BEAK Consultants, Ltd.	✔	✘	✘	
Biorenewal Technologies, Inc.	✔	✘	✘	
CET /Thorne Environmental Services, Inc.	✔	✘	✘	
Consulting Environmental Engineers, Inc.	✔	✘	✘	

Organization	Apply Bioremediation	Add Allochthonous Microorganisms	Supply Allochthonous Microorganisms	Comments
DuPont Remediation Services	✔	✘	✘	
E.V.S. Consultants	✔	✘	✘	
Ecology & Environment, Inc.	✔	✘	✘	
Enviros, Inc.	✔	✘	✘	
Geraghty & Miller, Inc.	✔	✘	✘	
Gesellscahft for soil & groundwater	✔	✘	✘	
Groundwater Technology, Inc.	✔	✘	✘	
InterPhase Environmental Inc.	✔	✘	✘	
IT Corporation	✔	✘	✘	
Jacques Whitford Environmental Ltd.	✔	✘	✘	
OHM Corporation	✔	✘	✘	
Texaco Environmental Services	✔	✘	✘	
Alpine Remediation Products	✘	✘	✔	Distributor for Reliance Biochemical.
Osprey Biotechnics	✘	✘	✔	
SBP Technologies, Inc.	✘	✘	✔	
Technical Resources, Inc.	✘	✘	✔	TRI has isolated a TCE-degrading microorganism for the EPA. They are not commercial distributors.
American Cyanamid, Lederle Laboratories	✘	✘	✘	
Bioprime Ltd.	✘	✘	✘	Bioprime produces a folic acid additive for secondary wastewater treatment.
Chandler	✘	✘	✘	Supply nutrient supplement
The BOC Group, Inc.	✘	✘	✘	
EIMCO Process Equipment Co.	✘	✘	✘	Manufacture bioreactor equipment
Exxon Bioremediation	✘	✘	✘	In-house bioremediation only
Frontier Refining, Inc.	✘	✘	✘	
Grace-Sierra	✘	✘	✘	Producer of controlled-release nutrient for microbial enhancement.

Table 2.1. (Continued)

Organization	Apply Bioremediation	Add Allochthonous Microorganisms	Supply Allochthonous Microorganisms	Comments
James River Corporation	✘	✘	✘	
Resource Analysts, Inc.	✘	✘	✘	
Shadrack Engineering Ltd.	✘	✘	✘	Represent companies marketing treatment systems.
Viridian Bioprocessing Ltd.	✘	✘	✘	Involved in biotreatment of liquid industrial effluents.

† - Indicates organizations which isolate microorganisms from contaminated sites, culture (with or without enhancing) the microorganisms and inoculate the microorganisms back to the site or any other site.

Table 2.2. Summary of Bioremediation Involvement of Responding Commercial Organizations

Breakdown of Bioremediation Involvement	*Organizations*		
	Total	International	Canadian
Number of Organizations Contacted	189	11	30
Number of Responding Organizations Involved in Bioremediation	53	4	10
Apply Bioremediation	41	3	9
Add Allochthonous Microorganisms	37	3	9
Supply Microorganisms	20	2	5
Supply Microorganisms - sole activity	20	2	3
	4	0	0

Note: International Organizations consists of non-Canadian, non-United States locations.

Table 2.3. Microbial Contents of Current Commercial Inocula

Company	Identification			Application	
	Genus species	Chemicals Treated	Source of Inoculum	On-site	*In situ*
Alko Biotechnology Ltd.	*Rhodococcus chlorophenolicus* Strain CP-2	Penta/Tetra/Tri and Dichlorophenol, Chlorocatechol, Guaiacols	Isolate (enriched) from chlorophenol contaminated environments	Compost piles, reactors, biofilters	
	Rhodococcus spp. CG-1	Polychlorophenols	Contaminated soil	as above	
	Mycobacterium spp. CG-2	Polychlorophenols	Contaminated soil	as above	
	Consortia	Oil contaminated solids	Enriched isolates from contaminated soils	Compost	Landfarming
Alpine Remediation Products (Distributor for Reliance Biochemical Corporation)	Unidentified consortia (RBC 212)	Gasoline, Diesel, Benzene, Toluene, Xylenes, Ethyl Benzene and Polyaromatic Hydrocarbons	Proprietary	All	All
	Unidentified consortia (RBC 216)	Petroleum Oil & Grease, Animal Fats & Oils, Triglycerides and Polyaromatic Hydrocarbons	Proprietary	All	All
	Unidentified consortia (RBC 218)	Heavy Petroleum Hydrocarbons, Phenol, Polyaromatic Hydrocarbons, Creosote and Pentachlorophenol Wastes	Proprietary	All	All
Argonne National Laboratories	*Phanerochaete chrysosporium*	Pentachlorophenol	ATCC	Bioreactor	
	Consortia	Diesel Fuel	Isolate from contaminated site	Bioreactor	Soil

Table 2.3. (Continued)

Company	Identification		Application		
	Genus species	Chemicals Treated	Source of Inoculum	On-site	*In situ*
Bioscience, Inc.	Consortia of: *Pseudomonas, Nocardia, Bacillus, Micrococcus,* etc. under tradename Microcat.	Petroleum Hydrocarbons, Solvents, Monomers & Pesticides	In-house supplier	Bioreactor, Sewage Treatment	Soil, Groundwater, Landfarming
BioTrol, Inc.	*Flavobacterium* spp.	Pentachlorophenol, Polyaromatic Hydrocarbons	In-house culture collections, ATCC	Yes	Yes
	Methylosinus trichosporum OB3b	Chlorinated Solvents (Trichloroethene)	In-house culture collections, ATCC	Yes	Yes
CRA Advanced Technical Development	*Nocardia* spp.	Diesel Fuels	House Culture Collection	Soil Bed	
Ebasco Environmental	Consortia	Petroleum Hydrocarbons	Treatment site	Bioreactor	
	Consortia	Polyaromatic Hydrocarbons, Aromatics (BTX)	Treatment site	Bioreactor	
Eco-Bac, Inc. (Distributor for Micro-Bac International, Inc.)	Unidentified (Micro-Bac)	Waste water influent	Proprietary	All	
	Unidentified (Mega-Bac)	Oil and Grease	Proprietary	Grease Traps	
	Unidentified (Para-Bac)	Paraffin	Proprietary		Oil wells
	Unidentified (M-1000H)	Acetone, Cresol, Ethanol, Methanol, Methylene Chloride, Polychlorinated Biphenyls (PCBs), Pentachlorophenol, Phenol, Benzene, Toluene, Trichloroethene, Trichloroethane, xylene and higher molecular weight alkanes	Proprietary		
EMCON Associates	Consortia	Petroleum Hydrocarbons	Isolates from soil		Landfarming

Table 2.3. (Continued)

Company	Identification		Chemicals Treated	Source of Inoculum	Application	
	Genus	species			On-site	*In situ*
EMCON (continued)	*Bacillus* spp.		Petroleum Hydrocarbons	Commercial supplier		Landfarming
Enviroflow, Inc.	*Bacillus subtilus* spp.		Petroleum Hydrocarbons Animal and Vegetable Fats Proteins, Carbohydrates Creosote Phenols Municipal Sewage	Proprietary	Yes	Yes
ESE Biosciences, Inc.	Consortia predominantly of *Pseudomonas*, *Micrococcus*, *Corynebacterium*, *Mycobacterium* and *Actinomycetes*.		Alcohols, Aromatics, Carbohydrates, Detergents, Ketones, Petroleum Hydrocarbons, Phenols, Phthalates, Solvents, Municipal Wastes	Contaminated Site isolates		Landfarming, Soil, Groundwater
Heritage Remediation Engineering	Consortia (proprietary mixtures of identifed microorganisms)		Methyl Ethyl Ketone Methyl Isobutyl Ketone Toluene	Municipal sewage treatment plant	Bioreactor	Groundwater, Soil
	Consortia (proprietary mixtures of identifed microorganisms)		Acrylic Acid	Industrial biological treatment plant		Groundwater, Soil
	Consortia (proprietary mixtures of identifed microorganisms)		Diesel Fuel	Polybac + Polybac-n nutrients	Bioreactor	Groundwater, Soil
	Consortia (proprietary mixtures of identifed microorganisms)		Diesel and Gasoline	Municipal sewage treatment plant	Bioreactor	Groundwater, Soil
Kiseki Inc.	Unidentified consortia (KBC 104)		High BOD cellulose	Commercial supplier	Sewage	
	Unidentified consortia (KBC 101, 102, 107, 109)		Polychlorinated Biphenyls (PCBs), Pentachlorophenols, Hydrogen Sulphide (H_2S), Phenols, Foams, Detergents, Oil & Grease, Cresol		All	All

Table 2.3. (Continued)

Company	Identification		Chemicals Treated	Source of Inoculum	Application	
	Genus	species			On-site	*In situ*
Kiseki (continued)	Unidentified consortia (KBC 100)		Hydrocarbons	Isolated from areas yielding hydrocarbon degraders - oil spills, salt lakes.		Oil wells
Merkert Laboratories Inc.	Unidentified (ML-21)		Hydrocarbons, Diesel Fuel Pentachlorophenols, Jet Fuel, Toluene, Ethylene Glycol, all organics except Polychlorinated Biphenyls	Commercial collection	All	All
	Unidentified (Bio-degreaser)		Fats, Oils, Greases	Commercial collection	All	All
Osprey Biotechnics	*Pseudomonas fluorescens*		Anthracene Benzene *o*-Chlorotoluene *p*-Chlorotoluene Cresols (mixed) Dinitrooctylpthalate Dichlorobenzene 1,1-Dichloroethane 1,2-Dichloroethane Dichloropropane Dichlorotoluene Ethylene glycol Pentachlorophenol Phenanthrene Phenol 1,1,2-trichloroethane	Proprietary	Yes	Yes

Company	Identification		Chemicals Treated	Source of Inoculum	Application	
	Genus	species			On-site	*In situ*
Osprey (continued)	*Pseudomonas putida*		*m*-Chlorotoluene Crude oil 2-Ethoxyethanol Ethylene glycol Ethylbenzene Isoprenoids Methyl ethyl ketone Methylene chloride Naphthalene Terpene 1,1,2,2-tetrachloroethane Toluene Waste Oils/Sludges, Xylene	Proprietary	Yes	Yes
Polybac Corporation	The components of Polybac products are: *Acinetobacter calcoaceticus* *Aspergillus oryzae* *Bacillus cereus* *Bacillus megaterium* *Bacillus subtilis* *Bacillus thuringiensis* *Micrococcus* sp. *Myxobacter* *Nitrobacter* sp. *Nitrosomonas* sp. *Saccharomyces cerevisiae* *Thiobacillus*		The types of wastes which can be remediated using formulations of these microorganisms include: Animal and poultry wastes, fibrous wastes, filamentous organisms, fats, oils and greases, oil field and refinery wastes, cellulose, lignins, pulp and paper wastes, ammonia, hydrocarbons in saline environments, hydrocarbons, phenols, polyaromatic hydrocarbons, aromatics, glycols, formaldehyde, waxes, cyanide, proteins, carbohydrates	Proprietary	All	All
ReTeC, Remediation Technologies, Inc.	*Phanerochaete chrysosporina*		Polyaromatic Hydrocarbons	Utah State University	Compost	Soil, Landfarming

Table 2.3. (Continued)

Company	Identification		Chemicals Treated	Source of Inoculum	Application	
	Genus	species			On-site	*In situ*
ReTeC (continued)	*Pseudomonas* spp.		Pentachlorophenol	Culture collection, in-house treatment site	Bioreactor	Soil, Landfarming, Groundwater
	Anaerobic consortium		DDT	Culture collection, in-house treatment site		Soil
	Pseudomonas spp.		Polyaromatic Hydrocarbons	Culture collection, in-house treatment site	Bioreactor	Soil, Landfarming
	Alcaligenes spp.		2,4-D, 2,4,5-T Chlorophenols	Culture collection, in-house treatment site	Bioreactor	Soil, Groundwater
	Pseudomonas spp.		2,4-D, 2,4,5-T	University collection	Bioreactor	Soil, Groundwater
	Consortia		Cyclo-alkanes, Oils	Culture collection, in-house treatment site	Bioreactor	Soil, Landfarming
SBP Technologies, Inc.	*Pseudomonas putida*		Creosote & related low molecular weight Polyaromatic Hydrocarbons	Soil sludge samples from contamiated sites	Yes	No
	Pseudomonas paucimobilis		Fluoranthene and other high molecular weight Polyaromatic Hydrocarbons	Soil sludge samples from contaminated sites	Yes	No
	Pseudomonas spp.		Pentachlorophenol	Soil sludge samples from contaminated sites	Yes	No
	Pseudomonas cepacia		Trichloroethene	TCE-contaminated sites	Yes	Yes

Table 2.3. (Continued)

Company	Identification		Chemicals Treated	Source of Inoculum	Application	
	Genus	species			On-site	*In situ*
Technical Resources, Inc.	*Pseudomonas cepacia*		Trichloroethene, Dichloroethene Toluene, Phenol *o*-, *m*-, *p*-Cresol *o*-, *m*-, *p*-Xylene Styrene Naphthalene Cumene Indole Anthranilic acid	Laboratory stock culture isolated from industrial waste treatment lagoon.	Fermentors	Groundwater, Soil
Waste Stream Technology, Inc.	*Pseudomonas putida*		Polyaromatics, Total Petroleum Hydrocarbons, Halogenated Organics, Fuel Oils, Jet Fuel	Site isolate		Landfarming
	Alcaligenes faecalis		Aromatics (BTEX), Gasoline, Volatile Organic Compounds (VOCs), Phenols	Site isolate	Bioreactor	Landfarming, Groundwater
	Pseudomonas spp. (fluorescent)		Jet Fuel, Diesel, Fuel Oil, Waste Oil, Phthalate Esters, Cutting Oil, Turbine Oil, PAHs	Site isolate		Landfarming
	Pseudomonas spp.		Aromatics (BTEX), Volatile Organic Compounds, Gasoline, Phenols, Pentachlorophenol	Site isolate	Bioreactor	Landfarming
	Arthrobacter spp.		Pentachlorophenol	Site isolate		Landfarming
	Pseudomonas fluorescens		Crude Oil, Fuel Oil, Diesel Fuel	Site isolate		Landfarming
	Pseudomonas fluorescens		Crude Oil	Marine isolate		Ocean
	Acinetobacter johnsonii genospecies 7		Crude Oil, Fuel Oil	Marine isolate		Ocean
	Psychrobacter spp.		Crude Oil, Diesel, Fuel Oil	Site isolate		Landfarming

Table 2.4. Ecological Profiles of the Microorganisms used in Current Commercial Inoculants (in Table 2.3).

Acinetobacter					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Gram-negative cocci Exhibit "twitching" motility Some strains may produce a capsule	Common water and soil microorganism Commonly found on skin Occasional animal parasite	Non-fermentative aerobic metabolism Dioxygenases	Utilize a restricted range of sugars Lignin (variety of dimers)	Aerobic metabolism	Implicated in nosocomial (hospital acquired) infections Penicillin resistant

Brock and Madigan, 1991.
Vasudevan and Mahadevan, 1991.

Actinomycetes					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Form a sub-branch of Gram-positive bacteria Great morphological variability; rods Filamentous, often branching growth, produce "microcolonies" Non-motile, non-sporeforming	Soil Isolated from teeth (animals, humans)			Most species facultative anaerobes Carbon dioxide required for maximum growth Optimum temperature 35-37°C Organic nitrogen required for growth	Many species are potential human pathogens Frequently infect face, neck, thoracic and abdominal sites Moderate to high susceptibility to many antibacterial drugs

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Table 2.4. (Continued)

Alcaligenes					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Rods, coccal rods, or cocci Gram-negative Motile with peritrichous flagella Nitrate reduced only to nitrite Some strains capable of aerobic nitrogen-fixation	Common saprophytic microorganism in soil and water Many strains isolated from clinical specimens	some strains capable of oxidizing hydrogen; prefer microaerophilic conditions characterized by limited action on carbohydrates may cause some confusion with *Pseudomonas*	May utilize carbon monoxide (CO) from incomplete combustion of hydrocarbons and catabolism of lignin Aerobic CO-oxidizing hydrogen members of genus; may represent significant sink for CO	Obligate aerobes Optimum temperature 20-37°C *A. faecalis* cannot grow anaerobically in the presence of nitrate	Occasionally cause opportunistic infections of man

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Table 2.4. (Continued)

Arthrobacter					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Growth cycle demonstrates two types of cell morphology Stationary cells are coccoid; exponential phase cells are irregular rods Gram-positive Nonmotile May be phylogenetically related to *Micrococcus*	Soil is usual habitat Less numerous in soils with increasing acidity Also isolated from fish, dairy waste-activated sludge and sewage	Respiratory metabolism; never fermentative Considerable enzymatic versatility	Do not hydrolyze cellulose Some strains decompose herbicides, caffeine, nicotine, phenol, and other unusual organic compounds	Obligate aerobes Do not survive 63°C for 30 min Optimum temperature 25-30°C Good growth on standard media at neutral pH Plates may require 2 week incubation period May require biotin as growth factor	Considered as non-pathogenic

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Table 2.4. (Continued)

Aspergillus					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Genus of the fungal group *Deuteromycetes* Common name for group - fungi imperfecti Eukaryotic cells Organotrophic Morphological structure composed of septate hyphae No sexual spores produced	Soil, decaying plant material, surfaces of animal bodies	Wide range of degradative enzymes Crucial role in mineralization of organic carbon Many extracellular enzymes produced	Used for the commercial production of citric acid Able to attack a number of plant polymers including cellulose Some species capable of antibiotic production	Carry out active metabolism when conditions are favourable; adequate moisture, aeration, and relatively high concentrations of usable substrates Spore formation permits survival during periods of dessication Generally tolerant of wide pH range May dominate in low pH environments Most species mesophilic; some members of genus are thermophilic	Can act as a plant pathogen Invasive to plant wall; causes fungal wilt infections Causative agent of animal infections such as ringworm, athlete's foot, and other dermatomycoses

Brock and Madigan, 1991.

Bacillus					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Gram-positive rods Form endospores Very resistant to adverse conditions	Most common organism to be isolated from soil or air Soil is primary habitat	Produce extracellular hydrolytic enzymes Potential GEM; transformation established in genus	Polysaccharides, nucleic acids and lipids Wide range of carbon sources Recently noted for ability to metabolize aromatic and hydroxyaromatic compounds	Aerobic or facultatively anaerobic Grow at low and high temperatures grow at high and low pH ranges Spores can survive in dormant state for many years Spores survive 80°C for 10 min Diversity of nutritional requirements and growth conditions; varies with species	Considered primarily as soil saprophytes; infect hosts only incidentally Few animal pathogens; causative agent of anthrax Problem with penicillin-resistant strains Cause two forms of food-poisoning Certain bacilli are insect pathogens; production of toxic protein

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Corynebacterium					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Irregular (pleomorphic) rods; often club-shaped Gram positive Difficult to assign aerobic strains to this genus; may be members of *Rhodococcus* Based on GC content, phylogenetically related to *Nocardia, Mycobaterium, Actinomycetes* and *Arthrobacter*	Common soil saprophyte	Oxidative degradation	Petroleum hydrocarbons, detergents Often effective in consortia of microorganisms	Most members of genus require specific nutrients such as vitamins, amino acids, purines and pyrimidines Facultatively anaerobic; some strains aerobic Moderately slow growth (1-2 days) Temperature range 30-37°C	Genus includes many animal pathogens; causative agent of diptheria Many plant pathogenic species

Bergey's Manual of Systematic Bacteriology, Vol. 2., 1986.
Brock and Madigan, 1991.

Flavobacterium					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Gram-negative rods Aerobic; strictly respiratory metabolism Non-motile; distinct from the gliding bacteria Oxidase-positive	Common in environmental habitats with a temperature range of 5 - 30°C Commonly isolated from aquatic habitats Some species are psychrophilic (grow at 0°C)	Noted for their capacity to degrade a wide range of natural carbon substrates Use respiratory pathways	May be found in habitats where cellulose - degrading gliding bacteria are common May act on smaller degradation products	Characterized by formation of yellow - orange pigmented colonies on particular substrates	Members of genera are non-hemolytic and have reduced potential as human pathogens Some strains have been isolated from medical specimens

Bergey's Manual of Systematic Bacteriology, Vol. 1., 1984.
Brock and Madigan, 1991.

Methylosinus					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Gram-negative Rods or vibrios Polar tuft flagellation Produces an exospore resting stage Produce complex intracytoplasmic membranes when grown on methane Type II membrane system; paired membranes running along the periphery of the cell	Widespread in nature in soil and water Found in methane rich muds, marshes, anaerobic zones of lakes, the rumen, and the mammalian intestinal tract Although they are obligate aerobes, they generally prefer microaerophilic conditions because of 0_2 sensitivity	Considered a methanotroph Use methane monooxygenase to oxidize methane Grow on some of the more oxidized one-carbon compounds, but also methane Assimilation of C-1 units by serine pathway; serine transhyroxymethylase	May oxidize higher hydrocarbons but not for growth (co-metabolism)	Growth on mineral salts with an atmosphere of 80 % methane and 20 % air Some strains may be thermophilic with optimum growth at 45-55°C	No evidence of pathogenicity A symbiotic association of type I methanotrophs and marine mussels reported

Bergey's Manual of Systematic Bacteriology, Vol. I., 1984.
Brock and Madigan, 1991.

Micrococcus					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Spherical cells; tetrads or irregular clusters Gram-positive Usually nonmotile	Primary habitat is mammalian skin Secondary habitat is meat and dairy products, soil and water	No noteworthy degradative capacities		Aerobic Strict respiratory metabolism Nutritional conditions vary with species; usually require complex media Grow well in the presence of up to 5 % NaCl Optimum temperature 25-37°C	Considered non-pathogenic Some strains can act as opportunistic pathogens Susceptible to a wide range of antibiotics

Bergey's Manual of Systematic Bacteriology, Vol. 2., 1986.
Brock and Madigan, 1991.

Mycobacterium					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Grouped as a separate family of bacteria Closely allied to the genera *Corynebacterium, Nocardia* and possibly the genus *Rhodococcus* Rod-shaped, non-motile cells Aerobic and slow growing May have branching or filamentous growth; no aerial mycelium Unique lipid content of cell wall (mycolic acids); property of acid-fastness	Many saprophytic species isolated from soil Pathogenic species also isolated from sites of infection	Mono-oxygenases Aerobic processes	Utilization of aliphatic hydrocarbons for growth Chlorophenols in contaminated groundwater	Slow growth of most strains due to hydrophobic nature cell wall Slow-growing strains require more than 7 days incubation at optimal temperature to produce colonies Cells strongly impermeable to nutrients Simple nutritional requirements Require selective isolation procedures from natural samples because of dominance of non-acid fast components of the population	Genus includes causative agents of tuberculosis and leprosy With large enough inoculum all mycobacteria produce granulomatus lesions in experimental animals Relatively resistant to broad-spectrum antibiotics (except streptomycin and rifampin)

Bergey's Manual of Systematic Bacteriology, Vol. 2., 1986.
Brock and Madigan, 1991.
Haggblom, M., and M. Salkinoja-Salonen, 1991.

Myxobacteria					
Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Group of bacteria exhibiting gliding motility; lack detectable locomotor organelles Motility in some genera due to presence of protein between cytoplasmic membrane and Gram-negative outer membrane Unicellular rods with tapering ends Cells aggregate to form fruiting bodies Form resting cells; myxocysts	Highest numbers found in cultivated soils Found in fresh water, soil, tree bark, dung of herbivores and in decomposing vegetation Some members found in rhizosphere of plants	Produce a wide range of extracellular enzymes Produce various carotenoid pigments Chemo-organotrophic	Capable of degrading proteins, nucleic acids, fatty acid esters and various polysaccharides Noted for production of soluble extracellular cellulases	Strictly aerobic Do not grow well > 40°C	May not be effective in mixtures because of capacity to produce antibiotics Capable of lysis of bacteria, yeast and certain filamentous fungi

Bergey's Manual of Systematic Bacteriology, Vol. 3., 1989.
Brock and Madigan, 1991.

Table 2.4. (Continued)

Nitrobacter					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Gram-negative Short rods Reproduce by budding Occasionally motile Cytoplasmic membrane system arranged as a polar cap Oxidize nitrite	Soil, fresh water, marine Sewage disposal systems Compost piles Aerobic environments where organic matter is being mineralized	Ability to utilize inorganic electron donor for energy; nitrite nitrite oxidase "True" nitrifying bacteria; produce nitrate	Effective nitrification of samples high in ammonia such as treated and untreated sewage effluent Important in environments where O_2 is available in interface with an anaerobic zone where ammonia may be high	Require aerobic conditions Nitrification process is much more extensive at neutral and alkaline environments Most nitrifying bacteria are inhibited by the presence of organic material under pure culture conditions Cannot be isolated on agar; need silica gel *Nitrobacter* may grow slowly with acetate as carbon source	Activities result in the acidification of the habitat due to accumulation of nitric acid Further nitrification is often inhibited in environments where the pH is low

Bergey's Manual of Systematic Bacteriology, Vol. 3., 1989.
Brock and Madigan, 1991.

Nitrosomonas					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Gram-negative Rods; grouped as short rods, pointed ends; long rods, rounded ends; long rods, rounded ends, chains Non-motile Peripheral membrane system oxidize ammonia	Soil, fresh water, marine Sewage disposal systems Aerobic environment wherever organic matter is being mineralized	Ability to utilize inorganic electron donor for energy; ammonia Mixed function oxygenase Ammonia - oxidizing bacteria; produce nitrite	Effective nitrification of samples high in ammonia such as treated and untreated sewage effluent Important in interfaces between aerobic and anaerobic zones high in ammonia	Require aerobic conditions Nitrification process is much more extensive at neutral and alkaline environments Optimum pH 5.8 - 8.5 Optimum temp. 5 - 30°C Most nitrifying bacteria are inhibited by the presence of organic material under pure culture conditions Cannot be isolated on agar; need silica gel	Activities result in the acidification of the habitat due to accumulation of nitric acid. Further nitrification is often inhibited in environments where the pH is low

Bergey's Manual of Systematic Bacteriology, Vol. 3., 1989.
Brock and Madigan, 1991.

Table 2.4. (Continued)

Nocardia					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Aerobic Have branching or filamentous (mycelial) growth; some aerial mycelium Mycelium fragments into rods and cocci Gram-positive to Gram-variable Most studies on metabolic pathways were carried out on strains now classified as rhodococci Phylogenetically related to *Mycobacterium*, *Corynebacterium* and other Actinomycetes	Widely distributed in nature Found in soil, water, air, sewage, clinical specimens, insects and plants Capacity to act as opportunistic pathogens	Wide range of oxygenases Some strains produce antibiotics (Nocardins, Cephalosporins)	Many strains able to utilize a variety of hydrocarbons Aerobic process Biodegradation of diesel fuel	Obligate aerobes Rate of growth for colony development 1- 5 days Enrichment techniques include provision of a hydrocarbon source such as mineral oil, plus ammonia	Genus includes causative agents of pulmonary, neural and/or systemic nocardiosis Lower animals such as dogs, goats, domestic and wild rodents etc., are susceptible to nocardial infection Nocardial plant pathogen causes galls in blueberry Resistant to penicillin Sensitive to antibiotics (aminoglycosides, tetracyclines, sulfonamides)

Bergey's Manual of Systematic Bacteriology, Vol. 2., 1986.
Brock and Madigan, 1991.

Phanerochaetes					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Major ecological group of fungi (Basidiomycetes) Known as "wood-rotting" or "white-rot" fungi Eukaryotic cell structure Cell mass composed of septate hyphae **Noteworthy Species:** *Phanerochaete chrysosporium* *Coriolus (Trametes) versicolor*	Soil Important role in decomposition of woody material in forests Capable of both cellulose and lignin degradation	Produce extracellular peroxidase enzyme systems Ligninases; lignin peroxidases Dechlorination of chloro-organic compounds Decolourization or elimination of chromophoric material	Lignin; complex polymers containing phenolic compounds Polycyclic aromatic hydrocarbons, polychlorinated biphenyls, hexachlorocyclohexane, dichloroaniline, pentachlorophenol Low and high molecular weight compounds in pulp bleaching effluents	pH 3.9 - 4.8 Growth under aerobic and anaerobic conditions Often require added C / N sources for bioremediation effect	Degradation of lignin derivatives ceases when fungus enters stationary phase Requires extension of life span of hyphae

Archibald *et al.*, 1990.
Brock and Madigan, 1991.
Gomez-Alarcon *et al.*, 1991.

Pseudomonas					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Straight or slightly curved rods Motile by means of polar flagella Gram-negative Some species produce fluorescent pigments Respiratory metabolism; never fermentative	Widely distributed in nature Soil, the surfaces of plants and animals	Catabolism of unusual organic substrates often requires activity of several different enzymes Important ecologically for acrobic degradation of soluble compound from plant and animal materials Use monoxygenases and dioxygenases to oxidize hydrocarbons Potential GEM; degradative traits on natural plasmids	Wide range of organic compounds used as carbon sources One of relatively few genera that can utilize hydrocarbons for growth Aromatic compounds degraded acrobically; effective use of mixed cultures of pseudomonads Anaerobic degradation of aromatic molecules by reductive process	Simple nutritional requirements Grow organo-trophically at neutral pH Grow at mesophilic temperatures Optimum 28°C	Some strains act as opportunistic pathogens Often associated with hospital acquired infections Difficult to treat with chemotherapy because of high incidence of antibiotic resistance Several plant pathogens

Bergey's Manual of Systematic Bacteriology, Vol. 1., 1984.
Brock and Madigan, 1991.

Rhodococcus					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Produce scanty mycelium No aerial mycelium Fragments into irregular rods and cocci Partially acid-fast Usually Gram positive Many of the transformations traditionally associated with nocardiae are due to rhodococci	Common soil microorganism Abundant in herbivore dung Also common in the gut of various insects Isolated from freshwater and marine habitats	Oxidative metabolism Similar to that of *Corynebacterium*, *Mycobacterium* and *Nocardia* Candidate for GEM with the development of gene cloning systems	Utilization of alicyclic hydrocarbons Also nitroaromatic compounds, polycyclic hydrocarbons, pyridine and steroids Breakdown of detergents and pesticides Implicated in breakdown of lignin and humic acid	Slow growth rate (1-3 days) Grow well on standard media and simple substrates Most strains grow between 15 and 40°C	Associated with pulmonary tuberculosis and bronchiectasis Causes infection in human patients compromised by immuno-suppressive drug therapy and/or lymphoma Some strains may act as plant pathogens

Bergey's Manual of Systematic Bacteriology, Vol. 2., 1986.
Brock and Madigan, 1991.

Table 2.4. (Continued)

Saccharomyces					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Genus of the fungal group *Ascomycetes* Common name of group - sac fungi Genus known as "yeasts"; unicellular fungi Spherical, oval morphology Reproduce by budding	Dominate in sugar-rich environments Natural habitats include fruits, flowers, tree bark	Utilize Kreb's cycle for aerobic respiration and utilization of sugars	Commercial application as baker's and brewer's yeasts	Cell material produced under aerobic conditions Fermentation processes for alcohol production use anaerobic conditions	Many species safe for consumption as component of food and beverage products

Brock and Madigan, 1991.

Thiobacillus					
Genus Description	**Habitat**	**Enzymatic Capabilities**	**Substrates / Applications**	**Culture Conditions / Limitations**	**Concerns**
Gram negative Polar flagellated rods Energy from oxidation of elemental sulfur, sulfides, thiosulfate	Ubiqnitous in nature Found in soil, freshwater, marine habitats where oxidizable sulfur is abundant	Sulfite oxidase pathway	Use elemental sulfur Can attach to crystals of metal sulfides	Strictly aerobic Some species have pH range of 6 - 8; others tolerate low pH of 1.5 - 4; 2- 5 Optimum temp. 20 - 43°C Can resemble pseudomonads by organotrophic growth in the presence of certain electron donors	Pure cultures are difficult to obtain Mixed cultures often contain organotrophic contaminants

Bergey's Manual of Systematic Bacteriology, Vol. 3., 1989.
Brock and Madigan, 1991.

Table 2.5. Examples of Chemicals Biotransformed by Microorganisms

Chemicals	Microorganisms	References
Acenaphthene	*Beijerinckia*	160
Acenaphthylene	*Beijerinckia*	160
2-Aminobenzoate	*Pseudomonas*	30
Anthracene	*Cunninghamella elegans*	43
Anthraquinone	*Pseudomonas paucimobilis*	118, 119, 214
Benz(a)anthracene	*Beijerinckia*	118
Benzoate	*Moraxella*	206
2,3-Benzo[b]fluorene	*Pseudomonas paucimobilis*	118
Biphenyls	*Pseudomonas paucimabilis*	121
Carbofuran	*Achramobacter*	18
Chloroaniline	*Pseudomonas*	97
Chlorobenzene	*Alcaligenes*, uncharacterized strains	97
4-Chlorobenzoate	*Acinetobacter, Arthrabacter*	97, 106
2-Chlorobenzoic acid	*Pseudomonas*	97
3-Chlorobenzoic acid	Anaerobic consortium, *Pseudomonas, Arthrobacter*	67, 97
4-Chlorobenzoic acid	*Arthrobacter, Nocordia, Pseudomonas*	97
2-Chlorobiphenyl (PCB)	*Rhizopus arrhizus, Phanerochaete chrysosporium*	16, 89, 214
4-Chlorobiphenyl	*Alcaligenes AS, Pseudomonas*	73, 214
Chlorolignin	*Phanerochaete chrysosporium*	40, 134
3-Chlorophenol	*Nocardia*	74, 75, 97
4-Chlorophenol	Consortium, anaerobic, *Alcaligenes, Flavobacter, Pseudomonas*	81, 89
Cholic acid	*Pseudomonas, P. putida*	165
Chrysene	*Pseudomonas paucimobilis*	118, 119
Coumaphos (pesticide)	*Flavobacterium*	18
Dibenzo-p-dioxin	*Pseudomonas* sp. strain HH69	61
Dibenzofuran	*Pseudomonas* sp. strain HH69	77
1,4-Dichlorobenzene	*Pseudomonas, Alcaligenes*	130
1,2-Dichloroethane	*Methanobacterium thermoaautotrophicum*	54
2,4-Dichlorophenoxyacetic acid (2,4-D)	Consortium, anaerobic, *Pseudomonas, Azotobacter,* Alcaligenes	97
DDT	*Phanerochaete chrysosporium*	40
Fluoranthene	*Pseudomonas paucimobilis*	118, 119
Fluorene	*Pseudomonas paucimobilis*	118
Lindane	*Phanerochaete chrysosporium*	40

Table 2.5. (Continued)

Chemicals	Microorganisms	References
Methyl alcohol	Unspecified organism, *Aureobasidium pullalans*	169
4-Methylbenz[a]anthracene	*Cunninghamella elegans*	41
4,4'-Methylene-bis-(2 chloroaniline)	*Bacillus megaterium, Nocardiopsis*	213
1-Methylnaphthalene	*Cunninghamella elegans*	42, 44
2-Methylnaphthalene	*Cunninghamella elegans*	42, 44
Naphthalene	*Pseudomonas paucimobilis*	118, 119
Nitroglycerin	Unspecified organism	190
Pentachlorophenol (PCP)	*Phanerochaete chrysosporium, Arthrobacter, Flavobacter, Coryneform,* Cladosporium, *Pseudomonas, Azotobacter vinelandii, Rhodococcus chlorophenolicus, R. CP-2*	16, 27, 40, 47, 55, 70, 119
Phenanthrene	*Pseudomonas paucimobilis, Cunninghamella elegans*	43, 118, 119
Phenol	*Aureobasidium pullulans*	119, 125
Phthalic acid	*Bacillus, B. brevis, Achromobacter*	2
Polychlorinated biphenyls (PCBs)	*Phanerochaete chrysosporium, Nocardia, Alcaligenes eutraphus Pseudomonas putida*	16, 40, 80, 107, 121, 211
Pyrene	*Pseudomonas paucimobilis*	118, 119
Radium	*Penicillium chrysogenum*, dead biomass	188
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	*Trichoderma viride, Pseudomonas putida, Bacillis megaterium Nocardiopsis, Phanerochaete chrysosporium*	10, 40, 141
Tetrachloroethane	*Desulfobacterium autotrophicum*	54
Tetrachloroethene	*Methanosarcina*	58
Toluene	*Pseudomonas putida F1, Pseudomonas,*	124, 125, 163
p-Toluene sulphonic acid	*Pseudo (comamonas) testosteroni*	99
1,1,1-Trichloroethane	*Desulfobacterium autotrophicum*	54
Trichloroethene	*Pseudomonas cepacia, Bacterium G4* (idfentified as *Acinetobacter*) *Pseudomonas putida, Escherichia coli, P. mendocina* (recombinant)	99, 123, 124, 125, 163
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	Consortium, anaerobic, *Pseudomonas cepacia, P. fluorescens*	67, 89, 97, 98, 156
2,4,6-Trinitrotoluene	*Phanerochaete chrysosporium*	190, 214
Xylene	*Pseudomonas*	110
Xylenels	*Arthrobacter, Flavobacterium*	119

Note: If no species name is given, the organism is an unidentified species of that genus.

Table 2.6 Survival of Allochthonous Microorganisms Added to Two Non-Contaminated, Amended Soils

Microorganism	Manured Soil			Manured and Limed Soil		
	Cell Number x 10^5 per gram dry soil					
Incubation (Days)	0	45	100	0	45	100
Penicillium spp.	24.7	7.7	7.1	33.9	2.7	2.2
Actinomycetes cellulosae	8.4	0.1	0.0	7.6	0.04	0.0
Bacillus cereus	23.2	57.4	49.3	86.9	8.6	12.3
Pseudomonas fluorescens	142.8	0.0	0.0	175	1.1	0.0
Azotobacter chroococcum	200	0.0	0.0	360	120	0.0

Adapted from Suflita, 1989b.

Table 2.7. List of Indigenous Microorganisms Detected in or Isolated from Contaminated Soil and/or Grounwater

Identification		Chemicals Present	Reference
Genus	species		
Acinetobacter anitratus		Nitroaromatics, Diesel, Gasoline	CET/Thorne
Actinomyces spp.		Diesel	CET/Thorne
Alcaligenes spp.		Phthalate esters	CET/Thorne
Alcaligenes denitrificans		Unleaded Gasoline	Ridgway *et al.*, 1990
Arthrobacter spp.		Gasoline, Mixed oils Hydrocarbons, BTEX, PAHs	CET/Thorne ADS Associes
Bacillus circulans		Mixed oils	CET/Thorne
Bacillus mycoides		Waste oil, Grease	CET/Thorne
Bacillus pumilis		Diesel	CET/Thorne
Bacillus lichenformis		Diesel	CET/Thorne
Bacillus cereus		Gasoline	CET/Thorne
Bacillus megaterium		Gasoline	CET/Thorne
Bacillus spp.		Hydrocarbon mixtures, BTEX, PAHs Mercury	ADS Associes Rochelle *et al.*, 1991

Table 2.7. (Continued)

Identification		Chemicals Present	Reference
Genus	species		
Brochothrix spp.		Diesel, Gasoline	CET/Thorne
Corynebacterium spp.		Gasoline, Diesel	CET/Thorne
		Hydrocarbons, BTEX, PAHs	ADS Associes
Enterobacter agglomerans		Diesel	CET/Thorne
Escherichia coli		Mercury	Rochelle *et al.*, 1991
Kurthia spp.		Mixed oil, Diesel	CET/Thorne
Lactobacillus spp.		Mixed oils	CET/Thorne
Micrococcus varians		Mixed oil	CET/Thorne
Micrococcus spp.		Hydrocarbons, BTEX to PAHs	ADS Associes
		Unleaded Gasoline	Ridgway *et al.*, 1990
Micrococcus roseus		Diesel	CET/Thorne
Micrococcus luteus		Gasoline, Diesel, Mixed oils	CET/Thorne
Nocardia spp.		Diesel	CET/Thorne
		Hydrocarbons, BTEX, PAHs	ADS Associes
		Unleaded Gasoline	Ridgway *et al.*, 1990
Oerskovia spp.		Hydrocarbon mixtures, BTEX, PAHs	ADS Associes

Table 2.7. (Continued)

Identification		Chemicals Present	Reference
Genus	species		
Pseudomonas	*pseudomallei*	Gasoline, Diesel	CET/thorne
Pseudomonas	spp.	Hydrocarbon mixtures, BTEX to PAHs	ADS Associes
Pseudomonas	*aeruginosa*	Nitroaromatics, Motor oil, Gasoline, Diesel Unleaded Gasoline Mercury	CET/Thorne Ridgway *et al.*, 1990 Tsai *et al.*, 1991
Pseudomonas	*cepacia*	Gasoline, Diesel, Mixed oils Unleaded Gasoline 2,4,5-T	CET/thorne Ridgway *et al.*, 1990 Steffan and Atlas, 1988, 1990 Steffan *et al.*, 1989
Pseudomonas	*maltophilia*	Unleaded Gasoline	Ridgway *et al.*, 1990
Pseudomonas	*alcaligenes*	Unleaded Gasoline	Ridgway *et al.*, 1990
Pseudomonas	*stutzeri*	Mixed oils, Phthalate esters Unleaded Gasoline	CET/Thorne Ridgway *et al.*, 1990
Pseudomonas	*pickettii*	Unleaded Gasoline	Ridgway *et al.*, 1990
Pseudomonas	*acidovorans*	Unleaded Gasoline	Ridgway *et al.*, 1990

Table 2.7. (Continued)

Identification		Chemicals Present	Reference
Genus	species		
Pseudomonas fluorescens		Gasoline, Diesel, Phthalates	CET/Thorne
		Unleaded Gasoline	Ridgway *et al.*, 1990
		Napthalene	Tsai *et al.*, 1991
		3-Chlorobenzoate	Fulthorpe and Wyndham, 1991
Pseudomonas luteola		Naphthalene	Tsai *et al.*, 1991
Pseudomonas putida		Gasoline, Diesel	CET/Thorne
		Unleaded Gasoline	Ridgway *et al.*, 1990
		Naphthalene	Sayler *et al.*, 1985
		Mercury	Tsai *et al.*, 1991
		3-Chlorobenzoate	McClure *et al.*, 1991
		Toluene	Sayler *et al.*, 1985
Serratia liquefaciens		Mixed oils	CET/Thorne

Table 2.8. Examples of Microorganisms That Have Been Genetically Engineered to Biodegrade Organic Contaminants

Microorganism	Plasmid Designation	Substrates Metabolized
Alcaligenes A5	pSS50	4-Clorobiphenyl
Alcaligenes spp. BR60	pBRC60	3-Chlorobenzoate
Alcaligenes xylosoxidans	dhlC	2,2-Dichloropropionate
Pseudomonas cepacia AC1100	RS1100-1	2,4,5-T
Pseudomonas convexa	PC1NIC	Nicotine, Nicotinic acid
Pseudomonas diminuta	pCS1	Parathion
Pseudomonas fluorescens V22	NAH	Naphthalene
P. fluorescens PR24B	pBRC60	3-Chlorobenzoate
Pseudomonas luteola V55	NAH	Naphthalene
Pseudomonas oleovorans	OCT	Octane, Decane
Pseudomonas putida	CAM	Camphor
P. putida R1	SAL1	Salicylate
P. putida PpG7	NAH	Naphthalene
P. putida PaW1	TOL	Xylene, Toluene
P. putida NCIB	pRA500	3,5-Xylenol
P. putida CINNP	pCINNP	Cinnamic acid
P. putida AC858	pAC25	3-Chlorobenzoic acid
P. putida ST	pEG	Styrene
P. putida RE204	pRE4	Isopropylbenzene
P. putida UWC1	pD10	3-Chlorobenzoate
Pseudomonas spp. E4	pUU204	2-Chloropropionic acid
Pseudomonas spp. B13	pB13	3-Chlorobenzoic acid
Pseudomonas spp. CF600	pVI150	Phenol
Pseudomonas spp. ASR2.8	pD10	3-Chlorobenzoate
Pseudomonas spp. C1T1	pC1T1	Aniline

Adapted from Trevors (personal communication).

Table 3.1 List of Responding Researchers and their Research Institutions

Research Institution	Contact
Applied Biotreatment Association	Dr. Tom Zitrides
Canberra College of Advanced Education	Dr. Alan Sheehy
Carnegie Mellon University	Dr. Richard Luthy
Centre for Groundwater Research	Dr. Douglas Mackay
Cornell University	Dr. Eugene Madsen
County of San Diego, Dept. Environ. Health Services	Mr. John Menatti
DVGW-Forschungsstelle, Institute der Universitat Karlsruhe	Dr. Peter Werner
Gouvernement du Quebec	Mr. Rock Begin
Industrial Biotechnology Association	Dr. Alan Goldhammer
Montana State University	Dr. Paul Sturman
National Institute of Public Health & Environmental Protection	Dr. Esther Soczo
Norwegian Geotechnical Institute	Dr. Gijs Breedveld
Rutgers University	Dr. Owen O'Connor
Rutgers University	Dr. Robert Ahlert
San Diego State University	Dr. Barbara Hemmingsen
Technical University of Braunschweig	Dr. H.H. Hanert
The John Hopkins University	Dr. Edward Bouwer
U.S. EPA, R.S Kerr Environmental Research Lab	Dr. Stephen Hutchins
University of California, Riverside	Dr. W.T. Frankenberger, Jr.
University of Illinois at Urbana-Champaign	Dr. Bruce Rittman
University of Karlsruhe	Dr. Bruno Herrling
University of Massachusetts	Dr. David Ostendorf
University of Michigan	Dr. Tim Vogel
University of Oklahoma	Dr. Joseph Suflita
University of Sheffield	Dr. A.J.M. Baker
University of Tennessee	Dr. Tom Phelps, Dr. David White
Utah State University	Dr. Ryan Dupont

Table 3.2. Summary of References Supporting Information Requirements

Information Requirement	References
Contaminant Mass and Distribution	
Source	29, 39, 50, 52, 64, 83, 91, 152, 164, 189, 192
Contaminant Mass	3, 28, 29, 48, 50, 53, 83, 91, 101, 103, 136, 151, 152, 164, 173, 184, 192, 193, 194, 195
Contaminant Attributes	
Chemical Structure	39, 110, 152, 175
Solubility	11a, 26, 39, 52, 91, 139, 152, 157, 158, 164, 179, 189, 202
Volatilization	37, 50, 52, 53, 65, 66, 83, 101, 126, 132, 164, 189
Sorption	3, 7, 8, 11a, 16, 31, 48, 55, 127, 152, 158, 164, 175, 188, 189, 196, 202
Chemical Reactions	50, 64, 87, 164
Biodegradability	
Biodegradation of Classes of Compounds	2, 4, 5, 7, 8, 29, 67, 80, 81, 88, 120, 152, 175, 181, 198
Analog Degradation	152
Cometabolism	31, 79, 85, 86, 129, 135, 152, 161, 164, 175, 191, 200, 207, 208
Kinetics	4, 90, 39, 63, 87, 132, 146, 147, 149, 151, 152, 158, 164
Smin, Secondary Substrate Utilization, Non-steady state growth	4, 94, 146, 147, 148, 149, 152
Toxicity	3, 12, 39, 53, 63, 91, 109, 111, 113, 126, 152, 154, 164, 173, 180, 185
Geochemistry	
pH	3, 11b, 28, 29, 39, 48, 50, 52, 53, 63, 126, 132, 151, 152, 158, 164, 173, 174, 184, 192, 193, 194
Eh/Redox	11a, 11b, 50, 53, 144, 164
Temperature	11a, 11b, 28, 29, 50, 52, 63, 114, 126, 151, 152, 158, 164, 173, 193, 194, 195
Osmotic Pressure	11b, 186

Information Requirement	References
Moisture Content	11b, 53, 63, 114, 132, 151, 158, 164, 173, 184
Nutrients	11a, 11b, 13, 23, 24, 28, 29, 39, 48, 50, 53, 63, 83, 91, 103, 114, 126, 151, 152, 158, 164, 167, 168, 173, 184, 193, 202, 204
Geophysical	
Delineation of Saturated & Unsaturated Zones	39, 50, 52, 64, 152, 164, 167
Hydraulic Conductivity	39, 48, 50, 64, 91, 152, 167
Hydraulic Gradient	39, 50, 64, 152
Porosity and Permeability	39, 48, 50, 52, 64, 91, 152, 184, 202
Cation Exchange Capacity	11b, 25, 48, 52, 64, 66, 132, 152, 184
Natural Organic Carbon	45, 50, 52, 64, 132, 167, 173, 184, 192, 195
Microbial Attributes	
Biomass	3, 15, 50, 63, 83, 91, 114, 132, 136, 143, 151, 167, 173, 175, 184, 193, 194, 202, 204
Community Composition	15, 50, 63, 89, 91, 118, 143, 151, 159, 173, 184, 193, 204
Activity/Growth	15, 50, 53, 63, 83, 91, 132, 136, 137, 143, 167, 193, 204
Distribution	15, 66, 72, 108, 133, 143
Use of Allochthonous Microorganisms	7, 9, 33, 53, 57, 71, 76, 78, 84, 92, 114, 117, 122, 131, 134, 137, 151, 175, 177, 178, 184, 193, 194, 195, 197
Treatability Studies - Microcosms/Columns	1, 15, 17, 28, 34, 48, 50, 52, 63, 69, 91, 103, 105, 112, 113, 136, 140, 152, 177, 179, 183, 202
Applications	
On-site Treatment	3, 37, 46, 49, 91, 104, 138, 151, 152, 182, 184, 193, 194, 195, 202
In situ Treatment	14, 15, 19, 32, 34, 35, 36, 38, 39, 41, 46, 50, 56, 59, 62, 91, 95, 101, 103, 105, 115, 116, 128, 150, 151, 152, 153, 155, 167, 173, 182, 191, 192, 201, 202, 203, 209, 210, 212

Table 3.3. **Influence of Increasing Substitution of Phenyldecanes on Microbial Growth**

Microorganism	1-Phenyldecane	1-Phenyl-4-methyldecane	1-Phenyl-4,4-dimethyldecane
Micrococcus cerificans	2	0	0
Pseudomonas aeruginosa	2	0	0
Mycobacterium phlei	2	2	0
M. fortuitum	2	2	0
M. rhodococcus	2	2	0
M. smegmatis	2	2	1
Nocardia opaca	2	2	1
N. rubra	2	2	1
N. corallina	2	2	1

Notes: 2 = Moderate or abundant growth
1 = Slight or questionable growth
0 = No growth

Adapted from Suflita, 1989a.

Table 3.4. **Influence of Halogen Type and Position on the Anaerobic Dehalogenation Rate of Monohalobenzoate by Dehalogenating Bacteria**

Position	Dehalogenation Rate (umoles/L/hr)			
	F	Cl	Br	I
Ortho	nd	0	1.20	1.20
Meta	0	4.63	3.70	0.89
Para	0	0	0.05	0.66

nd - not determined
Adapted from Suflita, 1989a.

Table 3.5 Selected Properties of Common Organic Contaminants

Organic Chemical	Density (g/cc)	Solubility (mg/L)	log K_{ow}	Henry's K (atm*m^3/mole)
Acenaphthene	1.189	3.93	4.07	$3.93\text{-}9.1^{-5}$
Acenaphthylene	0.899	3.93	3.92, 4.07	1.9^{-4} - 1.45^{-3}
Acetone	0.791	misc	-	-
Acetonitrile	0.79	-	0.34	-
Aldicarb	-	4000	0.70	-
Aldrin	-	0.01, 27-180	5.3, 3.01, 0.85	2.1^{-3}, 1.4
Anthracene	1.25	1.29, 0.073	4.45	8.6^{-5}
Anthraquinone	1.419	-	-	-
Benzene	0.879	1780	1.95-2.13	$2.67\text{-}5.3^{-3}$
Benz(a)anthracene	-	1.4^{-2}	5.61-5.9	1^{-6}
2,3-Benzo[b]fluoranthene	-	5.5^{-3}	6.06-6.57	-
Biphenyl	1.18	7.5-8.5	3.16-4.14	1.55^{-3}
Carbofuran	-	320	-	-
Chlorobenzene	1.106	488	2.18-3.79	$3.58\text{-}4.0^{-3}$
Chlorobenzoic acid	1.496-1.541	77-2100	1.98-2.69	-
Chloroform	1.489	8200	1.97	2.5^{-3}
2-Chlorophenol	1.241	28500	2.15-2.19	2.1^{-5}
Chrysene	1.274	254	2.0^{-3}-1.5	1.05^{-6}
Diazanon	-	53.5	-	-
Dichlorobenzene	1.288-1.458	49-133	3.38-3.56	$1.77\text{-}3.61^{-3}$
1,1-Dichloroethane	1.174	5500	1.79	$3.9\text{-}4.3^{-3}$
1,1-Dichloroethene	1.218	400	1.48	1.9^{-2}
1,2-Dichloroethane	1.25	9200	1.48	1.1^{-3}
1,2-Dichloroethene	1.27-1.29	600-6300	1.48, 2.09	2.7^{-3} - 7.2^{-2}
DDT	-	0.0034	3.48-5.13	$1.6\text{-}3.9^{5}$
Dieldrin	1.75	0.14	2.9-5.48	$1.7\text{-}4.57^{-7}$

Organic Chemical	Density (g/cc)	Solubility (mg/L)	log K_{ow}	Henry's K ($atm*m^3/mole$)
Fluoranthene	1.252	.265	, 4.9	6.5^{-6}
Fluorene	1.203	1.69-1.98	4.12-4.38	2.1^{-4}, 6.4
Heptachlor	1.579	0.056	3.87-5.44	$2.3\text{-}4.1^{-3}$
Hexane	0.660	13	4.11	-
Lindane	-	1.21-10	3.81, 3.89	1^{-6}
Methanol	0.796	-	0.64-0.82	-
Methylnaphthalene	1.02	27	3.87	-
Naphthalene	1.145	31.3-34.4	3.01-3.59	$3.6\text{-}4.6^{-4}$
Nitrobenzene	1.2	1900	1.85, 2.93	$1.1\text{-}1.31^{-5}$
Parathion	-	6.5-12.9	3.40	
Pentachlorophenol (PCP)	1.978	5.0	5.01-5.24	$2.1\text{-}2.8^{-6}$
Phenanthrene	1.025	1.6	4.46, 4.57	$1.3\text{-}2.3^{-4}$
Phenol	1.07	82000	0.22, 1.46, 2.2	$2.7\text{-}4.54^{-7}$
PCB 1242	1.35	0.13-0.34	4.11, 5.58	1.98^{-3}
PCB 1260	1.58	0.0027	6.11, 7.14	$6.1\text{-}7.1^{-3}$
Pyrene	1.277	0.032-0.14	4.88-5.96	5.1^{-6}
2,3,7,8-TCDD	-	0.2, 2^{-4}	6.84	2^{-3}
Tetrachloroethane	1.6	2900, 3000	2.56, 2.39, 4.9	$3.8\text{-}4.0^{-4}$
Tetrachloroethene	1.626	150-400	2.6, 2.88	1.2^{-2}
Tetrachlorophenol	1.6	-	4.10	-
Toluene	0.867	520	2.11-2.89	5.7^{-3}
1,1,1-Trichloroethane	1.35	4400, 5497	2.17-2.51	1.2^{-2}
Trichloroethylene	1.456	1100	2.29, 2.42	7.1^{-3}
Vinyl Chloride	0.9121	60	0.6, 1.23	1.9^{-2}
Xylenes	0.86-0.88	134-213	2.77-3.2	$5.27^{-3}\text{-}0.067$

Adapted from the Organic Properties Database, University of Waterloo by Permission of Dr. J. Barker.

Table 3.6 Environmental Degradation Half-Lives of Common Organic Contaminants

Organic Chemical	Unacclimated Aqueous Biodegradation Aerobic (hours)		Anaerobic (hours)		Photooxidation (hours)		Hydrolysis (hours)
	low	*high*	*low*	*high*	*low*	*high*	
Acenaphthene	295	2448	1180	9792	0.879	8.79	-
Acenaphthylene	1020	1440	4080	5760	0.191	1.27	-
Acetone	24	168	96	672	279	2790	No hydrolyzable groups
Acetonitrile	168	672	672	2688	1299	12991	>150,000 yrs
Aldicarb	480	8664	1488	15240	1	9.5	4580 days
Aldrin	504	14200	24	168	0.9	9.1	760 days
Anthracene	1200	11040	4800	44160	0.501	5.01	-
Benzene	120	384	2688	17280	50.1	501	-
Benz(a)anthracene	2448	16320	9792	65280	0.801	8.01	-
Benzo[b]fluoranthene	8640	14640	34560	58560	1.43	14.3	-
Biphenyl	36	168	144	672	7.8	110	No hydrolyzable groups
Chlordane	5712	33264	24	168	5.2	51.7	>197000 yrs
Chlorobenzene	1632	3600	6528	14400	72.9	729	>879 yrs
Chloroform	672	4320	168	672	623	6231	3500 yrs
Chrysene	8904	24000	35616	96000	0.802	8.02	-
Cresols	1	696	240	1176	1.1	16	No hydrolyzable groups
Dibenzofuran	168	672	672	2688	1.9	19	-
1,2-Dichlorobenzene	672	4320	2880	17280	152.8	1528	>879 yrs
1,1-Dichloroethane	768	3696	3072	14784	247	2468	-
1,1-Dichloroethene	672	4320	1944	4152	9.9	98.7	No hydrolyzable groups
1,2-Dichloroethane	2400	4320	9600	17280	292	2917	1.1 yrs
1,2-Dichloroethene	672	4320	2688	17280	25.2	286	-
DDT	17250	137000	384	2400	17.7	177	194000
Dieldrin	4200	25920	24	168	4	40.5	10.5 yrs

Organic Chemical	Unacclimated Aqueous Biodegradation Aerobic (hours)		Anaerobic (hours)		Photooxidation (hours)		Hydrolysis (hours)
	low	*high*	*low*	*high*	*low*	*high*	
Fluoranthene	3360	10560	13440	42240	2.02	20.2	-
Fluorene	768	1440	3072	5760	6.81	68.1	-
Heptachlor	360	1567	1440	6268	1	9.8	23.1
Malathion	100	1236	400	4944	1	9.8	8.8 yrs
Mecoprop	168	240	672	4320	3.8	37.8	-
Methanol	24	168	24	120	71	713	No hydrolyzable groups
Methyl parathion	360	1680	24	168	1	10.5	1728
Naphthalene	12	480	600	6192	2.96	29.6	-
Nitrobenzene	322	4728	48	300	0.544	5.44	-
Pentachlorophenol (PCP)	552	4272	1008	36480	139.2	1392	No hydrolyzable groups
Phenanthrene	384	4800	1536	19200	2.01	20.1	-
Phenol	6	84	192	672	2.28	22.8	No hydrolyzable groups
Pyrene	5040	45600	20160	182400	0.802	8.02	-
2,3,7,8-TCDD	10032	14160	40128	56640	22.3	223	-
1,1,1,2-Tetrachloroethane	672	4320	2688	17280	2236	22361	1604
Tetrachloroethene	4320	8640	2352	39672	384	3843	No hydrolyzable groups
Tetrachlorophenol	672	4032	2688	16128	364.4	3644	No hydrolyzable groups
Toluene	96	528	1344	5040	10	104	No hydrolyzable groups
1,1,1-Trichloroethane	3360	6552	13440	26208	5393	53929	0.73 yrs
Trichloroethylene	4320	8640	2352	39672	27	272	7704
Vinyl Chloride	672	4320	2688	17280	9.7	97	-
o-Xylene	168	672	4320	8640	4.4	44	No hydrolyzable groups
m-Xylene	168	672	672	12688	2.6	26	No hydrolyzable groups
p-Xylene	168	672	672	2688	4.2	42	No hydrolyzable groups

Note: Low and High refer to the lower and upper limits of the reaction rates. Low refers to the fastest half-life reported in the literature while high refers to the slowest half-life reported in the literature.

Adapted from Howard *et al.*, 1991.

Table 3.7. Biodegradation Pathways and Metabolic Products of the Major Contaminant Classes

Chemical Class	Examples	Biodegradative Pathways	Intermediates	End Products	Reference
Aliphatics					
1) Alkanes	Petroleum hydrocarbons ($CH_3(CH_2)_nCH_3$)	i) Terminal Methyl Oxidation	a) Primary alcohol ($CH_3(CH_2)_nCH_2OH$)	Beta oxidation products, TCA cycle products	Rittman *et al.*, 1991
			b) Aldehyde ($CH_3(CH_2)_nCOH$)	as above	
			c) Fatty acid ($CH_3(CH_2)_nCOOH$)	as above	
		ii) Subterminal Oxidation	a) Secondary alcohol ($CH_3(CH_2)_nCHOHCH_3$)	Beta oxidation products, TCA cycle products	Rittman *et al.*, 1991
			b) Ketone ($CH_3(CH_2)_nCO(CH_2)_nCH_3$)	as above	
		iii) Hydro-peroxidation	a) Fatty acid ($CH_3(CH_2)_nCOOH$)	Beta oxidation products, TCA cycle products	Mayfield (pers comm)

Table 3.7. (Continued)

Chemical Class	Examples	Biodegradative Pathways	Intermediates	End Products	Reference
		iv) Dehydrogenation	a) Alcohol ($CH_3(CH_2)_nCH_2OH$)	Beta oxidation products, TCA cycle products	Mayfield (pers comm)
			b) Aldehyde ($CH_3(CH_2)_nCOH$)	as above	
			C) Fatty acid ($CH_3(CH_2)_nCOOH$)	as above	
2) Alicyclics	Cyclohexane (C_6H_{12})	Hydroxylation	a) Alcohol ($C_6H_{11}OH$)	Beta oxidation products, TCA cycle products	Rittman *et al.*, 1991
			b) Ketone ($C_6H_{10}O$)	as above	
			c) Lactone ($C_6H_{10}O_2$)	as above	
			d) Adipic acid ($HOOC(CH_2)_nCOOH$)	as above	

Table 3.7. (Continued)

Chemical Class	Examples	Biodegradative Pathways	Intermediates	End Products	Reference
Polycyclics	DDT (p-$ClC_6H_4)_2CHCCl_3$	i) Reductive dechlorination	a) Lower chlorinated chlorophenyls (DDD, DDE, DDMU, DBP)	Lower chloorinated chlorophenyls which are highly recalcitrant	Atlas and Bartha, 1987
	Cyclodienes (*e.g.*, Aldrin, Heptachlor)	i) Epoxidation	a) Dieldrin, Heptachlor epoxide	Lower chlorinated cyclodienes which are highly recacitrant	Kearney and Kaaufman, 1971
Aromatics	Benzene (C_6H_6)	Ring oxidation followed by ortho or meta cleavage of catechol	a) Dihydrodiol ($C_6H_6(OH)_2$)	Beta oxidation products, TCA cycle products	Smith, 1990
			b) Catechol ($C_6H_4(OH)_2$)	as above	
	Alkyl Benzenes *e.g.*, Toluene ($C_6H_5CH_3$)	i) Oxidation of the methyl substituent followed meta cleavage of catechol (*P. aeruginosa*)	a) Benzyl alcohol ($C_6H_5CH_2OH$)	Beta oxidation products, TCA cycle products	Smith, 1990
			b) Benzyl aldehyde (C_6H_5CHO)	as above	
			c) Benzoic acid (C_6H_5COOH)	as above	
			d) Benzcarboxyhydrodiol ($C_6H_5(OH)_2COOH$)	as above	

Table 3.7. (Continued)

Chemical Class	Examples	Biodegradative Pathways	Intermediates	End Products	Reference
			e) Catechol ($C_6H_4(OH)_2$)	as above	
		ii) Ring oxidation followed by cleavage of catechol (*P. putida*)	a) Toluene dihydrodiol ($C_6H_5(OH)_2CH_3$)	Beta oxidation products, TCA cycle products	Smith, 1990
			b) 3-methyl catechol ($C_6H_3(OH)_2CH_3$)	as above	
Polyaromatics	Naphthalene	Oxidation	a) Naphthalene dihydrodiol	Beta oxidation products, TCA cycle products	Gibson and Subramanian, 1984
			b) 1,2-dihydroxynahpthalene	as above	
			c) Salicylic acid	as above	
			d) Catechol or gentisic acid	as above	

Chemical Class	Examples	Biodegradative Pathways	Intermediates	End Products	Reference
	Phenanthrene	i) Oxidation	a) phenanthrene dihydrodiol	Beta oxidation products, TCA cycle products	Gibson and Subramanian. 1984
			b) 1-hydroxy-2-napthoic acid	as above	
			c) O-phthalic acid	as above	
			d) Protocatechuic acid	as above	
		ii) Conversion to naphthalene	same as naphthalene	as above	Gibson and Subramanian, 1984
Halogenated Aromatics	Chlorobenzene (C_6H_5X)	i) Hydrolytic dehalogenation (nucleophilic substitution)	No detectable intermediates	C_6H_5B, X^- where B is NO_2, OH, COOH, CH_3	Rittman *et al.*, 1991
	Pentachlorophenol (C_6X_5OH)	ii) Reductive dehalogenation	a) Tetra-, tri-, dichloro quinones ($C_6X_4(OH)_2$)	CO_2, X^-, Benzene	Rittman *et al.*, 1991
			b) Alkylchlorobenzenes ($C_6(OH)_3H_2X$	as above	
	2-Fluorobenzoate (C_6H_4FCOOH)	iii) Oxidative dehalogenation	a) Catechol ($C_6H_4(OH)_2$)	TCA cycle products, X^-	Rittman *et al.*, 1991

Table 3.7. (Continued)

Chemical Class	Examples	Biodegradative Pathways	Intermediates	End Products	Reference
	4-Chlorocatechol (C_6H_4ClOH)	iv) Ring cleavage followed by dehalogenation	a) 4-Chlorocatechol ($C_6H_3(OH)_2Cl$)	TCA cycle products, X^-	Rittman *et al.*, 1991
			b) 3-Chloro-cis, cis mucconic acid ($C_6H_3(COOH)_2Cl$)	as above	
Halogenated Polyaromatics	Polychlorinated biphenyls (PCBs)	i) Dechlorination	a) Lower chlorinated biphenyls	Aromatic ring fission products	Gibson and Subramanian, 1984
			b) Hydroxybiphenyls	as above	
		2) Hydroxylation	a) Hydroxybiphenyls	Aromatic ring fission products	Gibson and Subramanian, 1984
Haligenated Aliphatics	Chloromethane (CH_3Cl)	i) Hydrolytic dehalogenation	a) Chloromethanol (CH_2ClOH)	Formaldehyde (CH_2O)	Rittman *et al.*, 1991
	Trichloroethylene (C_2HCl_3)	ii) Oxidation	a) TCE epoxide (C_2HCl_3O)	Acids - formic, glyoxylic, dichloroacetic	Rittman *et al.*, 1991
		iii) Reductive dechlorination	a) DCE ($C_2H_2Cl_2$) b) VC (C_2H_3Cl)	VC if cannot be degraded, ethene, methane, CO_2, Cl^-	Rittman *et al.*, 1991

Table 3.7. (Continued)

Chemical Class	Examples	Biodegradative Pathways	Intermediates	End Products	Reference
	1,2-Dichloroethane ($C_2H_4Cl_2$)	iv) Dihalo-elimination	a) Ethene (C_2H_4)	ethane, methane, CO_2, Cl^-	Rittman *et al.*, 1991

Table 3.8: **Concentrations of Selected Environmentally Important Metals That can be Tolerated by Naturally Occuring Microorganisms**

Antimony	100 nM	*Corynebacterium flaccumfaciens*
Arsenic	400 µg/mL	*Pseudomonas aeruginosa*
Sodium arsenite	1%	*Salmonella*
		Escherichia coli
		Proteus
		Shigella
		Klebsiella pneumoniae
Boron	5-50 Mm	*Pseudomonas aeruginosa*
Cadmium	200 µg/mL	*Staphylococcus aureus*
	400 µg/mL	*Pseudomonas aeruginosa*
	10 µg/mL	*Klebsiella aerogenes*
	1600 µg/mL	*Pseudomonas aeruginosa*
	50 µg/mL	*Klebsiella aerogenes*
Chromium	1600 µg/mL	*Pseudomonas aeruginosa*
	1500 µg/mL	*Pseudomonas fluorescens*
Cobalt	0.62 Mm	Soil microorganisms
	1.09 mM	Soil microorganisms
	100 mg/L	Heterotrophic bacteria
Copper	100 ppm	*Pseudomonas spp.*
	20 mM	*Escherichia coli*
	10 ppm	*Klebsiella aerogenes*
	0.68 mM	Soil organisms
	1.18 mM	Soil organisms
Lead	100 mg/L	Sediment bacteria
	3200 µg/mL	*Pseudomonas aeruginosa*
	50 µg/mL	Sediment bacteria
Mercury	100 µM	*Mycobacterium scrofulaceum*
	10 mg/mL	*Bacillus spp.*
		Pseudomonas fluorescens
		Pseudomonas sp.
Phenylmercuric acetate	0.3 ppm	*Pseudomonas sp.*
		Arthrobacter sp.
		Citrobacter sp.
		Enterobacter sp.
		Vibrio sp.
		Flavobacterium sp.
Molybdenum	100 mg/L	Sediment bacteria
	102 mg/L	*Pseudomonas aeruginosa*
Nickel	25 µg/mL	Sediment bacteria
Silver	100 mM	*Pseudomonas maltophilia*
		Staphylococcus aureus
Tellurium	64 µg/mL	*Pseudomonas aeruginosa*
Thallium	128 µg/mL	*Pseudomonas aeruginosa*
Tin	75 µg/mL	Sediment bacteria
Tungsten	102 µg/mL	*Pseudomonas aeruginosa*
Uranium	1600 µg/mL	*Pseudomonas aeruginosa*
Zinc	1600 µg/mL	*Pseudomonas aeruginosa*

Adapted from Trevors, 1991.

Table 3.9. Ranges and Optimums of Key Geochemical Parameters

Geochemical Parameter	Tolerable Range	Reference
Moisture Content	25-85% WHC, 0.01 MPa 60-80% WHC	Sims, 1990 Atlas & Bartha, 1987
Oxygen Content	Aerobic metabolism: >0.2 mg/L DO_2, minimum air filled pore spaces of 10% by volume. Anaerobic metabolism: O_2 concentration <1% by volume	Sims, 1990
Eh/Redox potential	Aerobes & Facultative anaerobes: > 50 mV Anaerobes: <50 mV	Sims, 1990
	Methanogenesis: -800 mV Sulfate reduction: -100 to -400 mV Metal reduction: +100 to +400 mV Nitrate reduction: +300 to -500 mV Aerobic respiration: +500 to +800 mV	Trevors, 1991
pH	5.5-8.5	Sims, 1990
Nutrients	Suggested C:N:P ratio of 120:10:1	Sims, 1990
	Phosphates >0.3 mg/L, Ammonia >1.0 mg/L	Kobus and Kinzelbach, 1989; Werner and Brauch, 1989

Geochemical Parameter	Tolerable Range	Reference
Temperature	Psychrophiles: 5-15°C (optimum <15°C) Mesophiles: 25-40°C (optimum 35°C) Thermophiles: 40-60°C	Atlas & Bartha, 1987
	Mesophiles: 15-45°C	Sims, 1990

WHC - Water Holding Capacity

Table 3.10. Terminal Electron Acceptors and Reaction Products of Selected Microbial Processes

Microbial Process	Terminal Electron Acceptors	Reaction Products	Microorganisms
Aerobic respiration	Oxygen	Water, CO_2, biomass	Strict or facultative aerobes
Methanogenesis	CO_2, HCO_3	CH_4	Methanogens
Sulfate reduction	SO_4^{2-}	SH^-, acetate	Various obligate anerobes
Sulphur respiration	S^o	S^{2-}	Few facultative and obligate anaerobes
Nitrate reduction	NO_3^-	NO_2^-, N_2O, N_2	Aerobes and facultative anaerobes
Metal reduction	MnO_2, Fe_2O_3	Mn^{2+}, Fe^{2+}	Aerobes
Fermentation	Organic carbon	H_2, CO_2, Organic acids	Various
Carbonate respiration	CO_2, HCO_3	CH_3COOH	Acetogenic bacteria
Fumarate respiration	Fumarate	Succinate	Succinogenic bacteria

Adapted from Trevors, 1991.

Table 3.11: Selected Properties of Several Clay Minerals and Organic Matter

Component	Cation Exchange Capacity me/100 g	Surface Area m^2/g	pH-Dependency of Charge	Colloidal Activity
Kaolinite	1-10	10-20	Extensive	Low
Montmorillonite	80-120	600-800	Minor	Extremely High
Vermiculite	120-150	600-800	Minor	High
Mica	20-40	70-120	Medium	Medium
Chlorite	20-40	70-150	Extensive	Medium
Allophane	10-150	70-300	Extensive	Medium
Organic Matter	100-300	800-900	Extensive	Medium

Adapted from Bohn *et al.* (1979).

Table 3.12: Effect of pH on Cation Exchange Capacity For 60 Wisconsin Soils

	CEC, me/100 g (MEANS FOR 60 SOILS)		
pH	Organic Matter	Layer Silicates	Whole Soil
2.5	36	38	5.8
3.5	73	46	7.5
5.0	127	54	9.7
6.0	131	56	10.8
7.0	163	60	12.3
8.0	213	64	14.8

Adapted from Bohn *et al.* (1979).

Table 3.13. Interpretations of Bioremediation Results Given Several Biomass Response Scenarios.

	Information Requirement	Conditions		Conclusions
		A priori (% of total population)	*Post Priori* (% of total population)	
Scenario A	Target Population Total Population Contaminant Concentration	10^2 (0.01%) 10^6 100 ug/g	10^4 (0.01%) 10^8 1 ug/g	Biodegradation is successful but is not likely due to the target microorganisms as the percentage of target to total microorganisms remained constant throughout biodegradation.
Scenario B	Target Population Total Population Contaminant Concentration	10^2 (0.01%) 10^6 100 ug/g	10^5 (0.1%) 10^8 1 ug/g	Biodegradation is likely due to stimulation of the target microorganisms.
Scenario C	Target Population Total Population Contaminant Concentration	10^2 (0.01%) 10^6 100 ug/g	10^6 (10.0%) 10^7 1 ug/g	Biodegradation is due to activity of the target microorganisms.
Scenario D	Target Population Total Population Contaminant Concentration	10^2 (0.01%) 10^6 100 ug/g	10^4 (0.01%) 10^8 90 ug/g	Biodegradation is not occurring perhaps as a result of toxicity to the indigenous microorganisms. Alternatively, the contaminant is very recalcitrant and biodegradation is possible but very slow.

Table 3.14. Comparison of Biomass and Activities of Microorganisms from Sediments Contaminated With Trichloroethene[1]

Depth (m)	[^{14}C] Acetate Incorporation into Lipids (dpm)2	[^{3}H] Thymidine Incorporation into DNA (dpm)[3]	Total PLFA pmol/g sediment (approx cfu/g sed.)	Trichloroethene Concentration (ug/Kg soil)
3	147	1690	8.97 (10^6)	-
	815	0		-
10	7560	0		-
	177	1930		-
	661	12200		670
20	675	4210		320
	2030	11300		190
	13100	0		250
30	131000	0		16300
	0	0		312400
	0	-		41800
	0	0		59400
40	0	393		361000
	0	0	6.90 (10^5)	373000
	292	0		38000
50	82	0		-
	166000	74100		-
	30400	1100	8.63 (10^6)	-
60	212000	35900		-
	2230	3790		-
	125	0		-
	53300	0		-
70	2270	1540		-
	253	20500		-
75	0	1960		-

[1] Table adapted from Major (1990). Data from Phelps *et al.* (1989)

[2] Incorporation of radiolabelled acetate into lipids expressed as dpm/day determined from duplicate time course experiments.

[3] Incorporation of radiolabelled thymidine into macromolecules expressed as above.

Table 4.1. Suggested Microcosm Designs for Evaluation of Several Bioremediation Strategies

Bioremediation Strategy	Contaminated Environment	Microcosm Design	Figure Number
In Situ	Saturated zone, groundwater and/or soil treatment	Static systems, no headspace	4.2a, 4.5
		Columns simulating flow conditions	4.6
In Situ	Vadose zone, soil treatment	Static systems with headspace	4.2a, 4.2b, 4.2c, 4.2d, 4.4
		Columns with air circulation through or over top of soil	4.6
Slurry Bioreactor	Anaerobic/aerobic, gas phase involved	Shaken flask system, gas circulated through mixture or over top of mixture	4.2b
		Simulated reactor with gas circulation	
Slurry Bioreactor	Anaerobic/aerobic, no gas phase involved	shaken flask with headspace	4.2b

Table 4.2. Characteristics of Laboratory-, Bench-, and Field-Scale (Pilot-scale) Treatability Studies

Scale	Type of Date Generated	Study Size	Time Required	Cost
Laboratory	Batch Kinetics	Beaker 1/100 Full Scale	Days/Weeks	$10,000 to $50,000
Bench	Mass Transfer Fluid Mobility Limitations	Bench-Top 1/10 Full Scale	Weeks/Months	$50,000 to $250,000
Field	Full Scale Design Performance, Cost Data	Full Scale	Months/Years	$250,000 $1,000,000

Adapted from Dupont, 1991

Table 4.3. Suggested Prioritization of Contaminants Occurring in Mixtures Based on Toxicity, Concentration, and Recalcitrance

Toxicity	Recalcitrance	Concentration	
		High	Low
High	High	3	2
	Low	3	2
Low	High	2	1
	Low	2	1

Rating system for Prioritization is as follows:

1 - Low priority, not necessary to target for treatment (depending on regulatory stringency)
2 - Medium priority, target for treatment and include in treatability studies
3 - High priority, design treatment systems and treatability studies around this contaminant

FIGURES

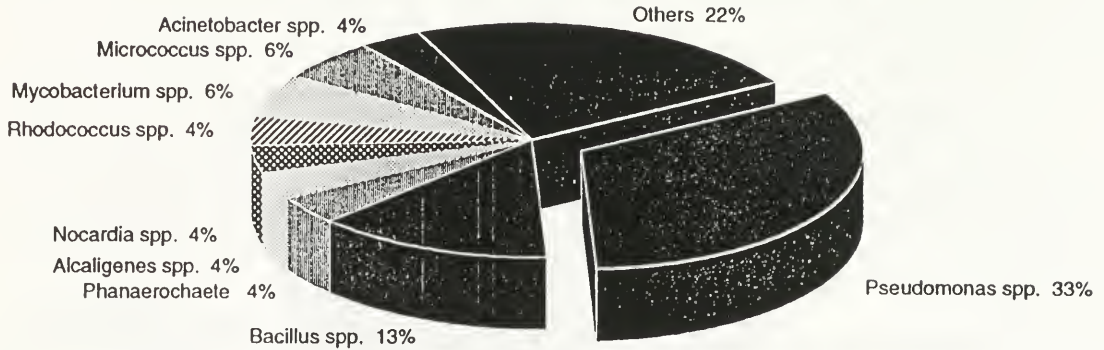


Figure 2.1. Frequency of Citation of Microorganisms in Commercial Inoculants

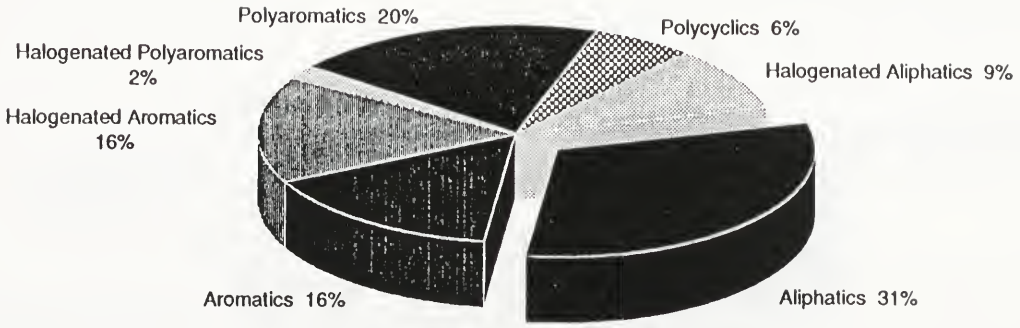


Figure 2.2. Frequency of Citataion of Chemical Classes Degraded by Commercial Inoculants

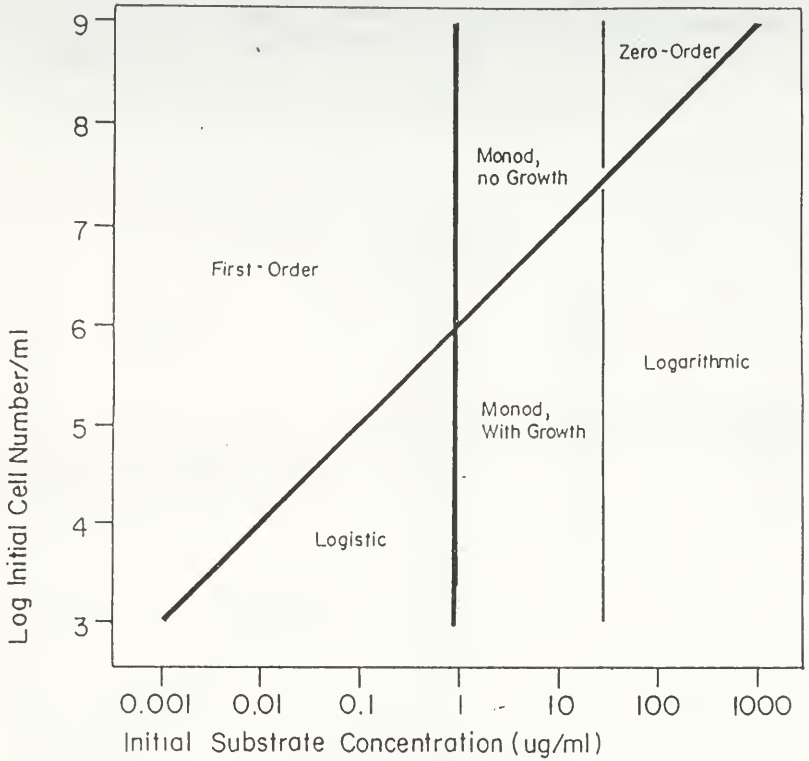


Figure 3.1a. ***Kinetic Models as a Function of Substrate Concentration and Bacterial Cell Density*** (Adapted from Alexander, 1985).

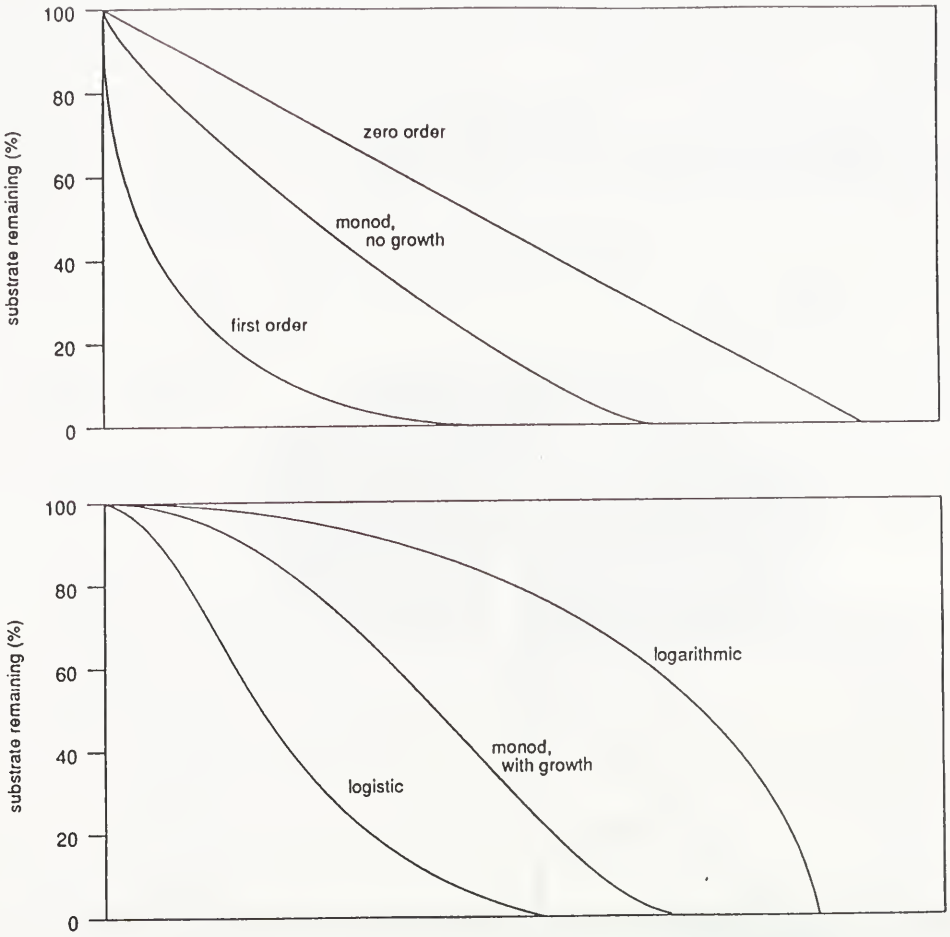


Figure 3.1b. ***Disappearance Curves of an Organic Compound Under Individual Kinetic Models*** (Adapted from Alexander, 1985).

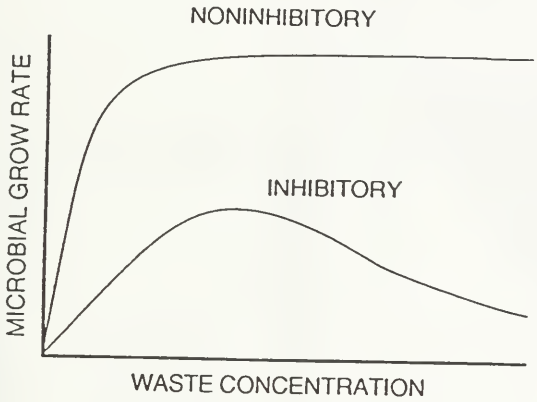


Figure 3.2. *Comparison of Microbial Growth Rate Versus Concentrations of Conventional (Non-Inhibitory) and Toxic (Inhibitory) Wastes* (Adapted from Rozich and Zitrides, unpubl).

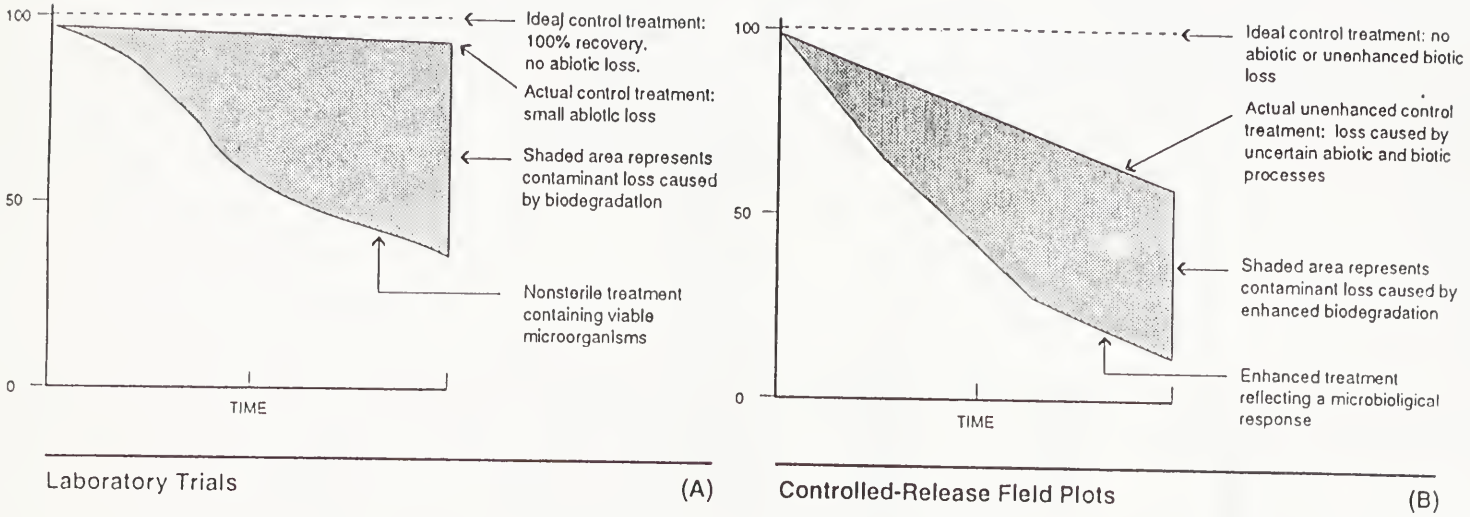


Figure 3.3. *Comparison of Biotic and Abiotic Mass Losses Observed in Typical Biodegradation Experiments Performed in (A) Laboratory Trials and (B) Controlled-Release Field Plots* (Adapted from Madsen, unpubl).

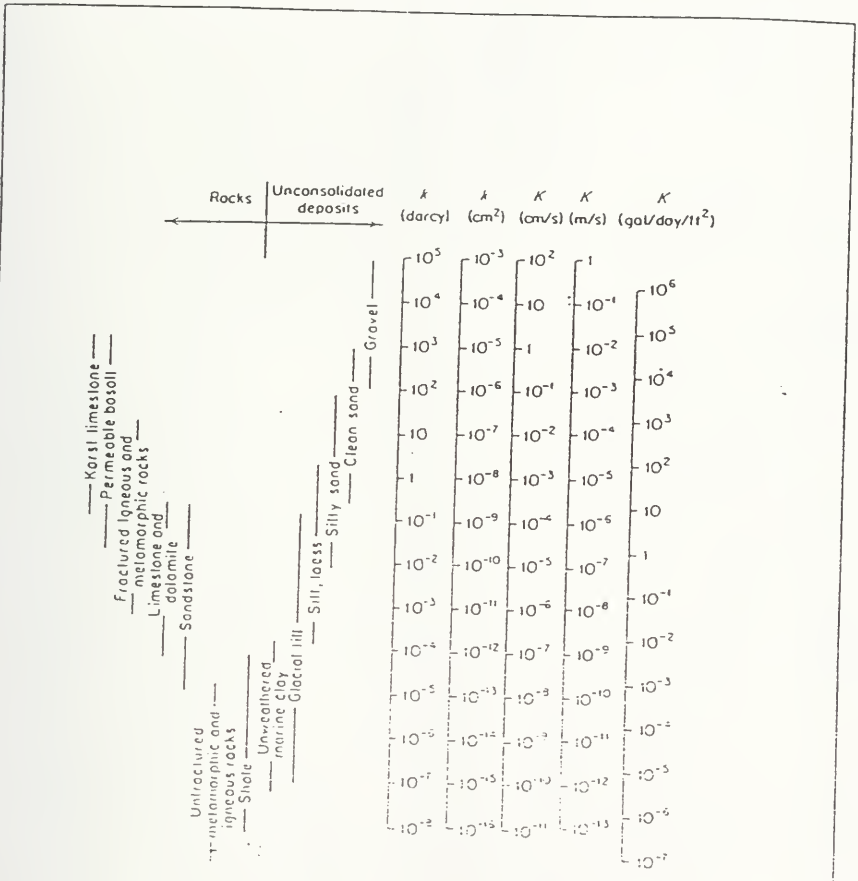
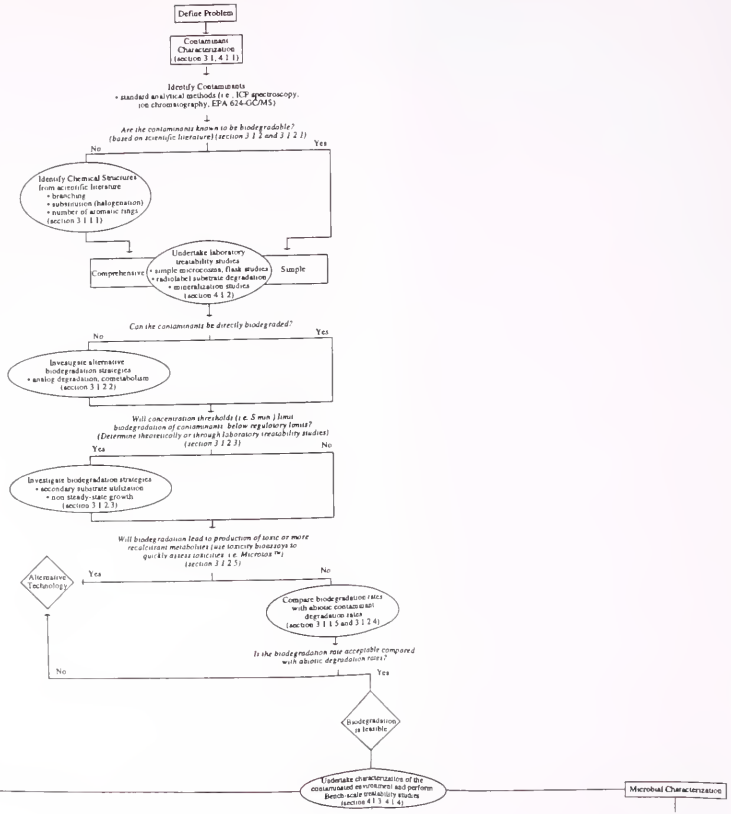


Figure 3.4. ***Range of Hydraulic Conductivities and Permeabilities of Geologic Deposits*** (Adapted from Freeze and Cherry, 1979).


Define Problem
Contaminant Characterization (section 3 1, 4 1 1)
Identify Contaminants
• standard analytical methods (i e , ICP spectroscopy, ion chromatography, EPA 624-GC/MS)
Are the contaminants known to be biodegradable? (based on scientific literature) (section 3 1 2 and 3 1 2 1)
No
Yes
Identify Chemical Structures from scientific literature
• branching
• substitution (halogenation)
• number of aromatic rings
(section 3 1 1 1)
Undertake laboratory treatability studies
• simple microcosms, flask studies
• radiolabel substrate degradation
• mineralization studies
(section 4 1 2)
Comprehensive
Sample
Can the contaminants be directly biodegraded?
No
Yes
Investigate alternative biodegradation strategies
• analog degradation, cometabolism
(section 3 1 2 2)
Will concentration thresholds (i e, 5 min) limit biodegradation of contaminants below regulatory limits? (Determine theoretically or through laboratory treatability studies) (section 3 1 2 3)
Yes
No
Investigate biodegradation strategies
• secondary substrate utilization
• non steady-state growth
(section 3 1 2 3)
Will biodegradation lead to production of toxic or more recalcitrant metabolites (use toxicity bioassays to quickly assess toxicities i e, Microtox™) (section 3 1 2 5)
Alternative Technology
Yes
No
Compare biodegradation rates with abiotic contaminant degradation rates (section 3 1 1 5 and 3 1 2 4)
Is the biodegradation rate acceptable compared with abiotic degradation rates?
No
Yes
Biodegradation is feasible
Physiochemical Characterization
Undertake characterization of the contaminated environment and perform bench-scale treatability studies (section 4 1 3 4 (4))
Microbial Characterization

CONTINUED ON OTHER SIDE

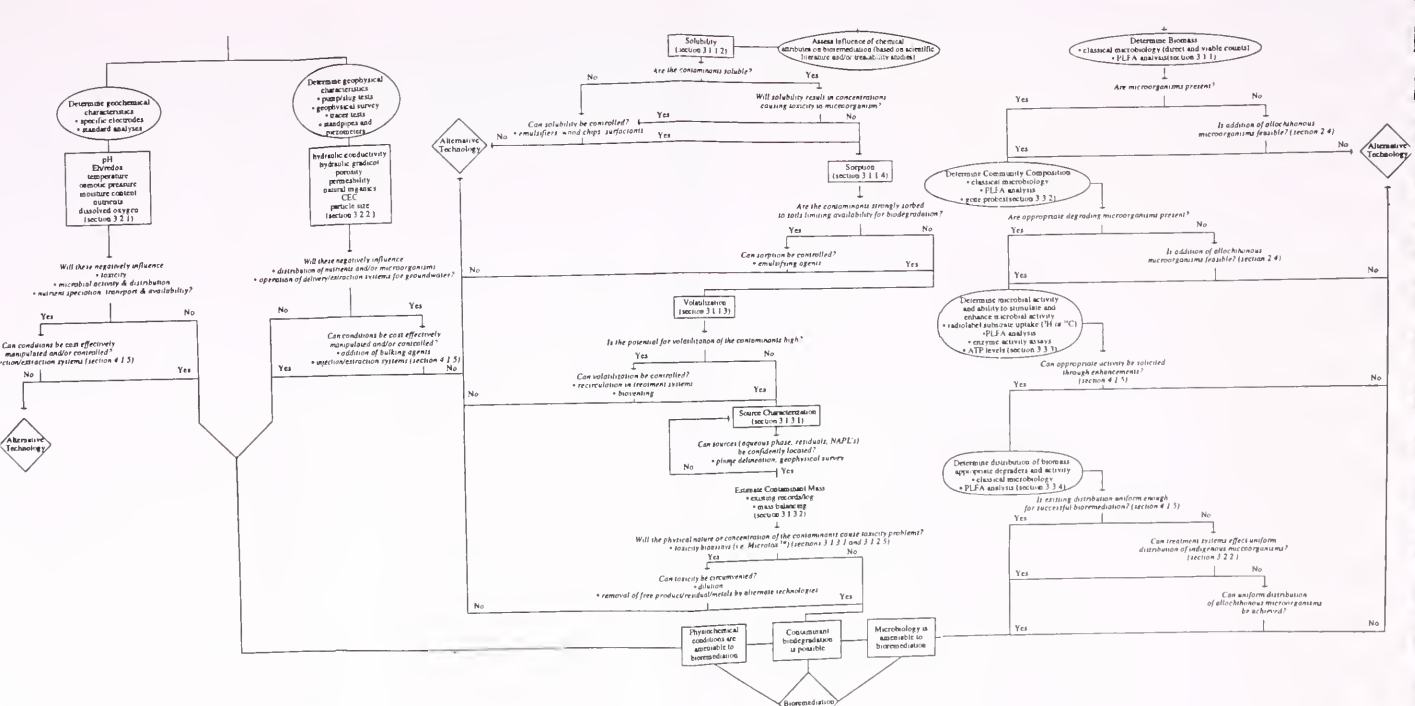


Figure 4 1 - Flowchart for Evaluation of Bioremediation Feasability

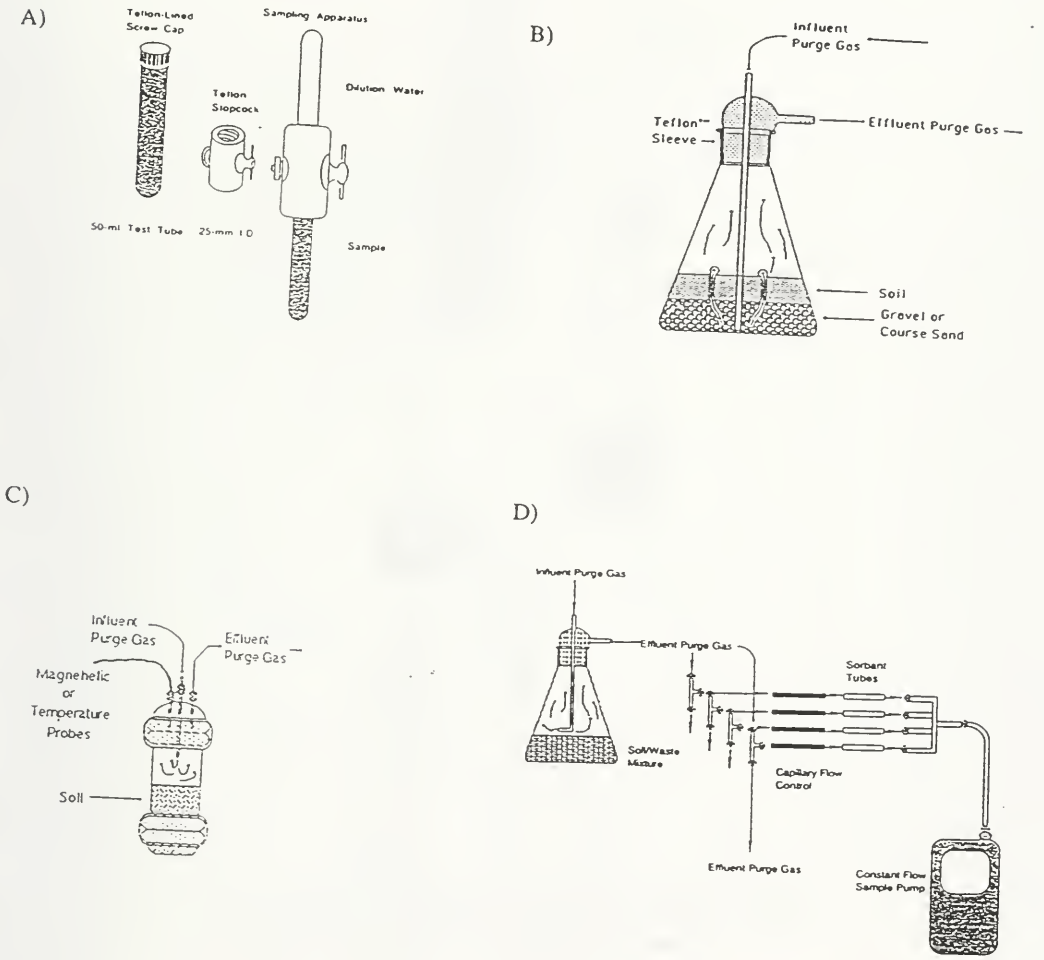


Figure 4.2. Laboratory Treatability Systems for Evaluation of Contaminant Biodegradability - (A) Simple test tube microcosm; (B) Laboratory flask apparatus for bioventing; (C) Laboratory column microcosm; (D) Sorbent tube sampling system. (Adapted from McFarland *et al.*, 1991).

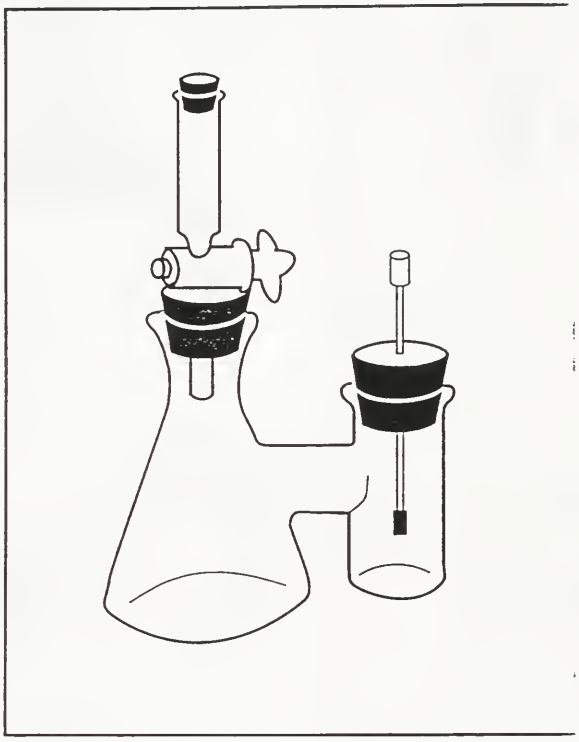


Figure 4.3: Biometry Flask for Mass Balance - Mineralizati n
Treatability Studies using Radiolabelled Substr s

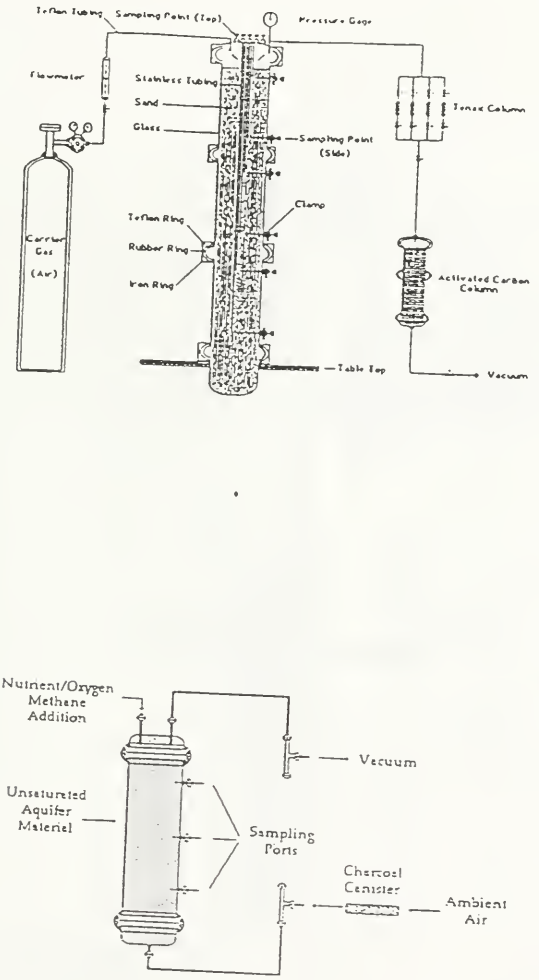


Figure 4.4. Bench-Scale Treatability Systems for Evaluation of the Potential for Bioremediation of the Vadose Zone via *In Situ* Bioventing (Adapted from Dupont, 1991).

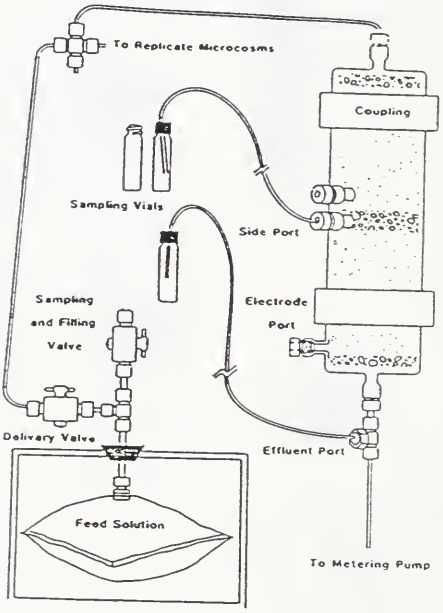


Figure 4.5. Bench-Scale Treatability System for Evaluation of the Potential for *In Situ* Bioremediation of Contaminated Groundwater (Adapted from Bengtsson *et al.*, 1985).

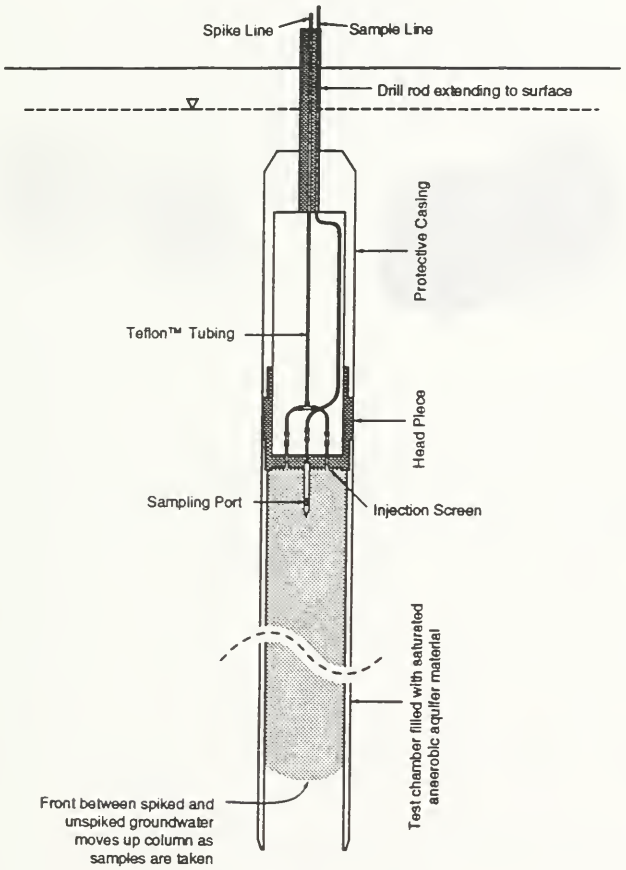

Spike Line
Sample Line
Drill rod extending to surface
Protective Casing
Teflon™ Tubing
Head Piece
Sampling Port
Injection Screen
Test chamber filled with saturated anaerobic aquifer material
Front between spiked and unspiked groundwater moves up column as samples are taken

Figure 4.6: *In Situ* Biodegradation Column Schematic (Adapted from Acton and Barker, 1991).

APPENDIX A

Glossary of Terms

GLOSSARY OF TERMS

Abiotic Reactions:	Any reaction that is not biologically-mediated. Those not encompassed by biotic reactions. These include chemical, phase transfer, photooxidation, photolysis and sorptive reactions.
Allochthonous:	Microorganisms which are not native to the environment to which they are added. Generally, microorganisms cultured and selected to possess desirable catabolic abilities are added to contaminated environments to provide enhanced microbial abilities.
Aquifer:	A geologic formation capable of yielding significant quantities of water when pumped. Generally, aquifers are composed of sands and gravels.
Aquitard:	A geologic formation incapable of yielding significant quantities of water and generally impermeable to water flow (*i.e.*, clays).
Beta Oxidation:	Metabolic process whereby long chain fatty acids are repeatidly shortened by two-carbon units. Shortened carbon units may then enter the tricarboxylic acid cycle (TCA cycle) to be converted to cellular building blocks (*i.e.*, succinate, pyruvate) and CO_2.
Bioaugmentation:	The addition of microorganisms "geared" to biodegrade specific contaminant(s). If supplied in sufficient numbers, these microorganisms may exhibit rapid initiation of bioremedial activity (ABTA, 1991).
Biotic Reactions:	Synonymous with biotransformation reactions (biodegradation, metabolism).
Biodegradation:	A subset of biotransformation in which the structure of an organic compound is completely mineralized to innocuous end products (*i.e.*, CO_2, water).
Bioremediation:	A managed or spontaneous process in which biological, especially microbiological, catalysis acts on pollutant compounds, thereby eliminating environmental contamination. Synonyms include biorestoration and bioreclamation (Madsen, 1991).

Biostimulation:	The addition of nutrients and/or electron acceptors to enhance the growth and accelerate the bioremedial activity of naturally-occurring (indigenous) microorganisms already present in an impacted matrix (ABTA, 1990).
Biotransformation:	The alteration of the molecular structure of a chemical by microbial (*i.e.*, enzymatic) catalysis. Alterations include condensation and mineralization reactions which change the number or complexity of intramolecular bonds. Alterations can be subtle, involving merely changes or loss of a substituent functional group (Madsen, 1991).
Biotreatment:	Methods of handling wastes and pollutant compounds through biological mediation. Includes *in situ* and on-site bioremediation, as well as off-site biological treatment at waste disposal and sewage treatment facilities.
Capillary Fringe:	The zone above the watertable which separates the saturated and the unsaturated (vadose) zones.
Carrying Capacity:	The sustainable size of a microbial population or community within a given environment or ecological niche.
Homeostasis:	A compensating mechanism that acts to maintain steady state conditions and, by a variety of control mechanisms, to counteract perturbances that would upset steady state (Atlas and Bartha, 1987).
In Situ Biodegradation:	The complete mineralization of an organic compound to innocuous end products, in its original place in the environment.
Microbial Metabolism:	The catalysis of organic compounds for subsequent use as growth substrates. Metabolism of pollutants can mean the biotransformation or the complete mineralization (biodegradation) of a compound.
Microhabitats:	Zones of varying of nutrient, pH, and Eh/redox conditions within soil clumps, pore spaces and fractures.
Mineralization:	Conversion of an organic molecule into its inorganic constituents (*e.g.*, CO_2, NO_3^-). The carbon derived from the mineralization reaction(s) is usually incorporated as cellular biomass.
On-site Biodegradation:	The complete mineralization of an organic compound to innocuous end products, in engineered above-ground treatment systems. Generally involves pump and treat and/or excavation technologies.

Pseudosolubilization:	Uptake through direct contact of cells with submicron sized droplets of the pollutant compound.
Residuals:	Contamination that does not solubilize and migrate in dissolved form but rather remains in free-phase sorbed to solid matrices.
Unbalanced Growth:	Microbial growth under conditions whereby carbon is available for growth but supplies of required inorganic nutrients are insufficient.

APPENDIX B

Letters and Questions Sent to Commercial
Organizations and Research Institutions

September 25, 1991 Beak Ref.:8007.1

title~. first~ last~
company~
street~
city~, state~
zip~
country~

Dear title~. last~:

Beak Consultants Limited (BEAK) has been contracted by the Ontario Ministry of the Environment (MOE) to assist in the development of a criteria document for bioreclamation. The essence of this document will be to identify information that can be used for evaluating bioreclamation proposals and verifying bioreclamation claims. The MOE is also interested in obtaining insight on how to justify the use, and evaluate the efficacy, of allochthonous microorganisms for *in situ* treatment of contaminated soil and groundwater, or their use for on-site treatment systems such as bioreactors and sewage treatment plants.

In particular, the MOE is seeking which specific endpoints must be collected (*e.g.* pH, moisture content, hydraulic conductivity, protozoan activity, enzymatic activity and gene expression, survival of allochthonous microorganisms) and what criteria should be used for evaluating the data (*e.g.* generally pH > 5.5; moisture content > 10%; hydraulic conductivity > 10^{-6} cm/s required for bioreclamation to proceed; biomass of allochthonous microorganisms must represent 10% of the active biomass; proof of survival and activity of introduced microorganisms). In addition, the MOE is interested in obtaining information on protocols that can be used to collect and evaluate the data.

I am writing you to request any information (reports, papers, presentation material) that you feel would assist the MOE in the development of this document. I would greatly appreciate a short note on what you believe are, or will be, key types of data for assessing bioremediation, and what developments you foresee in monitoring and evaluation protocols for bioreclamation.

All material used in the report will be duly referenced and contributors acknowledged.

Please forward the requested information to the above address. To meet the deadline for the report I would appreciate receiving your input by 10 August. Please feel free to solicit information or circulate this request to your colleagues if you feel they could make a valuable contribution. Do not hesitate to call if you have any questions. I thank-you in advance for your assistance.

Sincerely,
BEAK CONSULTANTS LIMITED

David Major, Ph.D.
Environmental Microbiologist

September 25, 1991 Beak Ref.:8007.1

title~. first~ last~
company~
street~
city~, state~
zip~
country~

Dear title~. last~:

The Ontario Ministry of the Environment (MOE) is interested in obtaining relevant information pertaining to the use of microorganisms for on-site or *in situ* bioreclamation. The information will be used to familiarize the MOE with the types and degradative capabilities of microorganisms that may be used in the Province of Ontario in the near future for site remediations or waste treatment. Beak Consultants Limited (BEAK) has been contracted by the Ontario Ministry of the Environment to assist in the collection of this information. Your firm has been identified as a commercial enterprise which may apply bioreclamation and may use allochthonous (non-indigenous) microorganisms for site remediation. On behalf of the MOE, we would appreciate if you could fill out the attached survey and return it to the above address. The information solicited is of a general nature and is not intended to solicit proprietary or confidential information.

The scope of information requested includes:

- use of allochthonous microorganisms by your organization;
- application of added microorganisms;
- genus and species used;
- chemicals and/or waste mixtures treated; and
- source of microorganism (e.g. in-house or external culture collection, cultured site microorganisms, sewage treatment plant, supplier).

In addition, I would appreciate any promotional and reference material that could be supplied by your organization. All material used in the report will be duly referenced and contributors acknowledged. To meet the deadline for the report I would appreciate your input by 10 August. Please do not hesitate to call if you have any questions regarding this project. I thank-you in advance for your assistance.

Sincerely,
BEAK CONSULTANTS LIMITED

David Major, Ph.D.
Environmental Microbiologist
Project Manager
encl.

SURVEY SHEET INSTRUCTIONS

Activity:

Please indicate if your organization has applied bioreclamation for site remediation or waste treatment and if allochthonous microorganisms have been used.

Identification:

Please identify the microorganism used under the genus and species column. If it is a consortia (mixture) of microorganisms, please indicate key species if possible or enter "Consortia" in the genus column.

Chemical:

Under the chemical column please list which compounds that the microorganism or consortia can biodegrade or bioaccumulate (please indicate which mechanism).

Source:

Under the source column please indicate the source of the microorganism or consortia (e.g. sewage treatment plant, isolated from treatment site, commercial or university culture collection, supplier). If the source information is not proprietary, please name the source or culture collection (e.g. ATCC, Bugs for Sale, Inc.).

Use:

Under this column enter how the microorganism(s) or consortia are used. On-site refers to above-ground, engineered systems. Under the on-site column, please select one or more of bioreactors, sewage treatment plants, septic systems, compost piles; In situ refers to applications which allow the wastes to be treated in place. Under the in situ column select one or more of groundwater, soil or landfarming. If the microorganisms can be used in all the applications listed in either column, enter "all" in the appropriate column.

SURVEY SHEET

Company Information

Corrected Information

Company:company~
Address:street~
city~
state~
zip~

Contact:title~. first~ last~

Phone: _____

Activity

Does your firm practice bioreclamation?: Yes No

Does your firm use allochthonous microorganisms?: Yes No

MICROBIAL DATA SHEET

Identification				Application	
Genus	species	Chemical Treated	Source of Inoculum	On-site	*In situ*

MICROBIAL DATA SHEET (Continued)

Please photocopy and attach additional microbial data sheets as required

Identification				Application	
Genus	species	Chemical Treated	Source of Inoculum	On-site	*In situ*

APPENDIX C

List of Bioremediation Organizations Contacted

Commercial Organization	Contact	Location
3M	Mr. Mohamed Elnabarawy	St. Paul, MN
ABB Environmental Services, Inc.	Mr. C.K. Taur	Camarillo, CA
Acadia Consultants & Inspectors Limited	Ms. Nichole LeBlanc	Moncton, NB
ADS Associes	Mr. Rene Fontaine	Montreal, QB
Advanced Bioremediation Services, Inc.	Mr. Warren Reynolds	Carlsbad, CA
Aer-O-Flo Environmental, Inc.	Mr. Emmi Schneider	Burlington, ON
Alko Biotechnology Ltd.	Dr. Risto Valo	Rajamaki, FINLAND
Allied-Signal Research & Technology	Mr. Stephen Lupton	Des Plaines, IL
Alpha Environmental Biosystems, Inc.	Mr. W.H. Worley	Lee's Summit, MO
Alpine Remediation Products	Mr. Glen White	Turlock, CA
Alton Geoscience	Mr. Jeffrey Wiegand	Irvine, CA
American Cyanamid, Lederle Laboratories	Mr. Joel Jerome	Boundbrook, NJ
American Petroleum Institute	Mr. David Chen	Washington, DC
Amoco Oil Company	Mr. Carl Olson	Chicago, IL
Applied Biotreatment Association	Ms. Katherine Devine	Washington, DC
Applied Geosciences, Inc.	Ms. Ann Baugh	Tustin, CA
Arco Pipeline Company	Mr. Steve Petersen	Independence, KS
Argonne National Lab.	Ms. Fran Clarke	Argonne, IL
Artic Slope Consulting Group	Ms. Rena Alexander Anderson	Anchorage, AK
Astre Corporation	Mr. Roy Ackerman	Charlotteville, VA
Atlantic Environmental Services, Inc.	Ms. Carol Roberson	Colchester, CT
AWD Technologies, Inc.	Mr. Gene Cutler	Houston, TX
Barr Engineering Company	Mr. Gary Hokkanen	Minneapolis, MN
BASF Corp.	Mr. Brian Diepeveen	Parsippany, NJ
Battelle	Mr. Robert Olfenbuttel	Columbus, OH
Battelle Pacific Northwest Laboratories	Mr. Dave Eakin	Richland, WA
Battelle, Pacific Northwest Laboratories	Ms. Terri Stewart	Richland, WA
Beck	Mr. Dave Ward	Port Coquitlam, BC
Bio-Care, Inc.	Mr. M.B. McMahon	San Jose, CA
Bio-Rem Canada Co.	Mr. Lothar Rabstein	Belwood, ON
Biodetox GmbH	Mr. Hein Kroos	WEST GERMANY
BioFiltration, Inc.	Mr. Dave Bare	Tampa, FL

Commercial Organization	Contact	Location
Biogenie, Inc.	Mr. Benoit Cyr	Quebec City, QB
Biopaq Lavalin	Mr. Derk Maat	London, ON
Bioprime Ltd.	Mr. Richard Akerboom	Norwich, VT
Biorecovery Systems, Inc.	Mr. Dennis Darnell	Las Cruces, NM
Bioremediation, Inc.	Mr. Richard Cassin	San Diego, CA
Biorenewal Technologies, Inc.	Mr. Allen Dines	Madison, WI
Bioscience, Inc.	Dr. Tom Zitrides	Bethlehem, PA
Biosystems Inc.	Dr. Micheal Lee	Chester Township, PA
Biothane Corporation, Anaerobic Process Section	Ms. Jill Jordan	Camden, NJ
Biotreatment Limited	Mr. Peter Barrett	Cardiff, Wales, UK
BioTrol, Inc.	Mr. Jeffrey Peltola	Chaska, MN
Biovac Environmental Services	Mr. Mark Lynch	St. Charles, MO
Biutec Biotechnologie	Mr. Helmut Effenberger	Vienna, AUSTRIA
BP Research	Mr. Keith Cowey	Cleveland, OH
BRGM, Departement Environnement & Risques	Mr. Hubert Bonin	Orleans, FRANCE
Burlington Environmental	Ms. Felicia Rein	Berkeley, CA
Cape Cod Research, Inc.	Mr. Brian Dixon	Buzzards Bay, MA
Cascade Earth Sciences, Ltd.	Mr. Les Tipton	Albany, OR
Celgene Corp.	Mr. Domenic Paone	Warren, NJ
CET Environmental Services, Inc	Mr. Keith Kaufman	Long Beach, CA
CH2M Hill	Mr. Duane Hicks	Bellevue, WA
Chemical Waste Management	Mr. Alan Li	Geneva, IL
Clifton Associates Ltd.	Mr. Dave Kent	Regina, SK
Conoco, Inc.	Mr. Ralph Beeman	Ponca City, OK
Consulting Environmental Engineers, Inc.	Mr. Mark Franson	W. Hartford, CT
CRA Advanced Technical Development	Dr. Peter McAllister	Roseville, NSW, AUSTRALIA
CRA Bioremediation	Dr. Bruce Kelley	North Sydney, NSW, AUSTRALIA
Dames & Moore	Mr. Tim Holbrook	Denver, CO
Danco Distribution Inc.	Mr. Charles Gauthier	Edmundston, NB
Dearborn Chemical Company, Ltd.	Mr. Alan Seech	Mississauga, ON
Delta Environmental Consultants, Inc.	Mr. Bill Newman	St. Paul, MN
Dorr-Oliver Incorporated	Mr. Timothy Bezler	Milford, CT

Commercial Organization	Contact	Location
Dow Chemical - Midland Division	Mr. Matt Davis	Midland, MI
Du Pont Environmental Remediation Services	Mr. R.L. Raymond Jr.	Aston, PA
Du Pont Environmental Remediation Services	Mr. Michael Lee	Aston, PA
Dynamic Corporation/RSKERL	Mr. Kevin Rumery	Ada, OK
E.I. Du Pont de Nemours & Co., Inc.	Mr. Scott Cunningham	Newark, DE
E.V.S. Consultants	Mr. Peter Nix	North Vancouver, BC
EA Engineering Science & Technology, Inc.	Ms. Gloria McCleary	Sparks, MD
Ebasco Environmental	Dr. Arthur Chu	Lyndhurst, NY
Eckenfelder, Inc.	Mr. Robert Norris	Mahwah, NJ
Eco-Bac inc.	Mr. Warren Brower	Calgary, AB
Ecology & Environment, Inc.	Mr. Gary Haden	Overland Park, KS
Ecology & Environment, Inc.	Mr. Jon Sundquist	Lancaster, NY
Ecolotrol	Mr. Roger Owens	Westbury, NY
Ecova Corporation	Mr. Bill Mahaffey	Redmond, WA
EG&G Idaho, Inc.	Mr. Patrick Dugan	Idaho Falls, ID
ELMCO Process Equipment Co.	Mr. Gunter Brox	Salt Lake City, UT
Ekokem Oy Ab	Mr. Sakari Salonen	Riihimaki, FINLAND
Elf Aquitaine, c/o Atochem	Mr. Alain Drexler	Somerville, NJ
Eli Lilly & Company	Mr. Stanley Parka	Indianapolis, IN
EMCON Associates	Dr. William Woods	San Jose, CA
Engineering Enterprises Inc.	Mr. Roy Murphy	Norman, OK
Engineering-Science, Inc.	Ms. Julia Kennedy	Cuyahoga Falls, OH
ENSITE, Inc.	Mr. Andrew Autry	Tucker, GA
ENSR Consulting & Engineering	Mr. Joseph Aiken	Westmount, IL
Enviro Search	Mr. Richard Kelly	Salt Lake City, UT
Enviroflow, Inc.	Mr. Frank Gregorio	Manassas, VA
Envirogen, Inc.	Mr. George Skladany	Lawrenceville, NJ
Environmental Microbiology Associates, Inc.	Ms. Katherine Baker	Newark, DE
Environmental Remediation, Inc.	Ms. Kathryn Purcell	Walnut Creek, CA
Environmental Resources Management, Inc.	Mr. Phillip Ludvigsen	Exton, PA
Enviros, Inc.	Mr. Leo Lehmicke	Kirkland, WA
ERC Environmental	Mr. David Bjostad	San Diego, CA

Commercial Organization	Contact	Location
ESE Biosciences, Inc.	Mr. Brian Hicks	Raleigh, NE
Exxon Bioremediation	Mr. Rory Davenport	Washingto, DC
First Environment, Inc.	Mr. George Kehrberger	Riverdale, NJ
Formula IV Corporation	Mr. Scott Miller	Scottsdale, AZ
Frontier Refining, Inc.	Mr. Rob Groenewold	Cheyenne, WY
Gas Research Institute	Mr. Tom Hayes	Chicago, IL
GE Corporate Research & Development	Mr. Herman Finkbeiner	Schenectady, NY
General Environmental Science	Dr. Frank Horsfall	Beechwood, OH
General Physics Environmental Services Corporation	Dr. Dennis Lew	Columbia, MD
Geobac Technology Group, Inc.	Mr. Victor Nowicki	Fredericton, NB
Geraghty & Miller, Inc.	Mr. Tom Crossman	Tampa, FL
Gesellschaft for Soil and Groundwater Remediation	Mr. Edward Durbek IV	Kirchheim, WEST GERMANY
Gore & Storrie Ltd.	Sir/Madam	North York, ON
Gosling Cubak Associates	Mr. Richard Raetz	Traverse City, MI
Grace-Sierra	Mr. Rick Carr	Cambridge, MA
Griffen Remediation Services	Ms. Diane Kroplin	West Milton, OH
Groundwater & Oil Recovery Systems, Inc.	Mr. Michel Boyer	Mississauga, ON
Groundwater Cleanup Inc.	Mr. Ted Keffer	Lancaster, PA
Groundwater Decontamination Systems, Inc.	Dr. Vidyut Jhaveri	Paramus, NJ
Groundwater Technology Inc.	Mr. Ronald Hicks	Concord, CA
Groundwater Technology, Inc.	Mr. Scott Wilson	Concord, CA
Groundwater Technology, Inc.	Mr. Mike Osborne	Mississauga, ON
Groundwater Technology, Inc.	Dr. Maureen Leahy	Wallingford, CT
H. Strauss Associates Inc.	Mr. Harlee Strauss	Natick, MA
Harding Lawson Associates	Mr. Donald Smallbeck	Novato, CA
Henkel Research Corporation	Mr. Sam Hom	Santa Rosa, CA
Heritage Remediation Engineering, Inc.	Mr. Kevin Reinhard	Romeoville, IL
Heritage Remediation/Engineering	Mr. Ed Copeland	Indianapolis, IN
Horsehead Resource Development Co.	Mr. John Oyler	Palmerton, PA
Hunter/Keck	Mr. James Quince	Williamston, MI
Hydro Group	Mr. Frank Lenzo	Bridgewater, NJ
ICI BioProducts & Fine Chemicals	Mr. Neil Gray	Mississauga, ON

Commercial Organization	Contact	Location
In-Situ Fixation Company	Mr. Tom Kochis	Chandler, AZ
Innovative Waste Technologies, Inc.	Mr. Robert Riseman	Kansas City, MO
International Technology Corporation	Mr. Paul Bay	Irvine, CA
International Technology Corporation	Mr. James King	Knoxville, TN
InterPhase Environmental, Inc.	Mr. Tony Bode	San Diego, CA
Intertox America	Mr. Stephen Robinson	Deer Park, TX
Jacques Whitford Environment Ltd.	Mr. Lloyd Lemon	Fredricton, NB
James Hill Corporation	Mr. Alan Prouty	Camas, WA
John Mathes & Associates, Inc.	Mr. Richard Bell	Columbia, IL
Keystone Environmental Resources, Inc.	Mr. Chris Steffy	Monroeville, PA
Kiseki Inc.	Mr. Mark Bishop	Calgary, AB
Lancy International Inc.	Mr. Chris Steffy	Warrendale, PA
Law Environmental, Inc.	Mr. George Wessman	San Diego, CA
Mantech Environmental Technology	Mr. Hubert Attaway	Panama City, FL
McLaren-Hart	Mr. Dennis Dineen	Irvine, CA
Merkert Laboratories Inc.	Dr. Clarence Baugh	Deerfield Beach, FL
Michigan Biotechnology Institute	Mr. Bob Hickey	Lansing, MI
Micro-Bac International Inc.	Mr. Sam Humphrey	Austin, TX
Microlife Technics	Mr. Milton Schwartz	Sarasota, FL
Monenco Consultants Ltd.	Mr. Dennis Trotter	Calgary, AB
Monsanto Environmental Science Corporation	Mr. Bryan Hopkins	St. Louis, MO
Montgomery Engineering, Inc.	Mr. Richard Jekins	Pasedena, CA
National Environmental Remdiation, Inc.	Mr. Alex Petroski	Folcroft, PA
NIPER	Ms. Rebecca Bryant	Bartlesville, OK
Niyp & Moore	Mr. David Richter	San Diego, CA
O'Connor Associates Environmental Inc.	Mr. James Carss	Calgary, AB
Ogden Environmental Services	Ms. Brenda Anderson	San Diego, CA
OHM Corporation	Mr. Paul Flathman	Findlay, OH
OHM Remediation Services of Canada Ltd.	Mr. Derk Maat	Oakville, ON
Oryx Energy Company	Mr. Jay Eubanks	Oklahoma City, OK
Osprey Biotechnics	Mr. John E. Hill	Sarasota, FL
P.J. Hannah Equipment Sales Corp.	Ms. Nancy Mounsteven	Mississauga, ON

Commercial Organization	Contact	Location
Polybac Corporation	Dr. David Magniarini	Bethlehem, PA
Quontime	Mr. Thomas Huhnold	Scarborough, ON
Remediation Technologies	Mr. Thomas Clark	Concord, MA
Remediation Technologies, Inc.	Dr. Gaylen Brubaker	Chapel Hill, NC
Remediation Technologies, Inc.	Dr. Hans Stroo	Seattle, WA
Resource Analysts Inc.	Mr. David Pease	Hampton, NH
RMI, Inc.	Mr. Mike Hendson	Greenville, SC
RMT	Mr. Gero Leson	Santa Monica, CA
Roberson Environmental Services Ltd.	Mr. Peter Roberson	Otterburn Park, QB
SAIC	Ms. Barbara Fairbanks	San Diego, CA
Sakota Environmental Technology Ltd.	Mr. Hanns Schnepf	Salzburg, AUSTRIA
SBP Technologies, Inc.	Ms. Heather Ford	South Hamilton, MA
SBP Technologies, Inc.	Mr. John Martiniere	Stone Mountain, GA
Sevenson Environmental Services, Inc.	Mr. George Torrance	Niagara Falls, NY
Shadrack Engineering Ltd.	Mr. Neil Pohl	Scarborough, ON
Shell Canada Products Ltd.	Ms. Lynn Calder Starr	Don Mills, ON
Shell Oil Co.	Mr. Curtis Stanley	Houston, TX
SNC, Inc.	Ms. Dana Mourato	Montreal, QB
Solmar Corp.	Sir/Madam	Oranos, CA
Strata Technology	Ms. Sandy Eccker	Monrovia, CA
Sussex Environmental Services, Inc.	Mr. Jeff Tucker	London, ON
Sybron Chemicals Inc.	Mr. Gary Hater	Birmingham, NJ
Technal Inc.	Mr. Arthur Gardner	Grand Junction, CO
Technical Resources, Inc.	Dr. Malcolm Shields	Gulf Breeze, FL
Texaco Environmental Services	Mr. Tony Palagyi	Universal City, CA
Texas Research Institute, Inc.	Ms. Frances Lueb	Austin, TX
The Boc Group, Inc.	Mr. J.F. Rakszawski	New Providence, NJ
The Traverse Group	Mr. Bill Korreck	Traverse City, MI
The Valspar Corporation	Mr. Paul Robinson	Minneapolis, MN
Toxic Treatments Inc.	Dr. Phil LaMori	San Francisco, CA
TreaTek	Mr. Anthony Ying	Grand Island, NY
U.S. Environmental Services, Inc.	Mr. Glenn Crum	Fort Worth, TX

Commercial Organization	Contact	Location
Unocal	Ms. Lori Patras	Brea, CA
V.F.L. Technology Corp.	Mr. Steven Kinback	Malvern, PA
Viridian Bioprocessing Limited	Dr. Chris Knowles	Chestfield, Whitstable, Kent, UK
Wastestream Technology Inc.	Dr. Brian Schepart	Buffalo, NY
Western Environmental Remediation, Inc.	Mr. Kent Baugh	Walnut Creek, CA
Woodward-Clyde Consultants	Dr. Michael Piotrowski	San Diego, CA
WW Engineering & Science	Mr. Mark Etringer	Grand Rapids, MI
Zimpro/Passavant Inc.	Mr. A.R. Wilhelmi	Rothschild, WI

APPENDIX D

List of Research Organizations Contacted

Reasearch Institution	Contact	Location
Ajou University	Dr. Soon Cho	Suwon, SOUTH KOREA
Alberta Environment, Industrial Waste Branch	Dr. Silver Lupul	Edmonton, AB
Alberta Environmental Center	Dr. Steve Davies	Vegreville, AB
Applied BioTreatment Association	Dr. Louise Nadim	Washington, DC
Argonne National Laboratory	Mr. Rajagopalan Venkatadri	Argonne, IL
California Department of Health Services	Mr. John Wesnousky	Sacramento, CA
California Dept. of Health, Alternative Technology Toxic Substances Program	Mr. Tony Luan	Sacramento, CA
Cambridge Analytical Association	Dr. Sam Fogel	Boston, MA
Canberra College of Advanced Education	Dr. Alan Shechy	Belconnen, A.C.T., AUSTRALIA
Carnegie Mellon University	Dr. Richard Luthy	Pittsburgh, PA
Center for Chemical Hazard Assessment	Mr. Philip Howard	Syracuse, NY
Centre for Groundwater Research	Dr. Douglas Mackay	Menlo Park, CA
Clemson University	Dr. Thomas Overcamp	Clemson, SC
Cornell University	Dr. James Gossett	Ithaca, NY
Cornell University	Dr. Eugene Madsen	Ithaca, NY
County of San Diego, Dept. Environmental Health Services	Mr. John Menatti	San Diego, CA
CSIRO	Dr. Chris Barber	Wembley, WESTERN AUSTRALIA
David Taylor Research Centre	Dr. Paul Schatzberg	Annapolis, MD
Dearborn Chemical Company, Ltd.	Mr. Alan Seech	Mississauga, ON
Delft University of Technology	Dr. W.L. Dalmijn	Delft, THE NETHERLANDS
Department of Energy	Dr. Rashalee Levine	Germantown, MD
Drexel University	Dr. N. Nirmalakhandan	Philadelphia, PA
DVGW-Forschungsstelle am Engler-Bunte-Institute der Universitat Karlsruhe	Dr. Peter Werner	Karlsruhe, GERMANY
Gouvernement du Quebec	Mr. Rock Begin	Sainte-Foy, QB
Hong Kong Polytechnic	Dr. N.F.Y. Tam	Hung Hom, HONG KONG
Illinois Institute of Technology	Dr. Glenn Paulson	Chicago, IL
Industrial Biotechnology Association	Dr. Alan Goldhammer	Washington, DC
Institute of Gas Technology, Biotech. & Env. Research	Mr. V.R. Srinivasan	Chicago, IL
Kassel University	Dr. Wolfgang Schafer	Kassel, WEST GERMANY
Lawrence Livermore National Laboratory, Environmental Restoration Division	Ms. Paula Krauter	Livermore, CA
Louisiana State University	Dr. Gary Miller	Baton Rouge, LA
Lund University	Dr. Thomas Welander	Lund, SWEDEN

Research Institution	Contact	Location
Maine Department of Environmental Protection	Mr. Jim McMillan	Augusta, ME
McGill University	Dr. Raymond Yong	Montreal, QB
McMaster University	Dr. M.H.I. Baird	Hamilton, ON
Michigan Biotechnology Institute	Dr. Howard Ratcliffe	Lansing, MI
Michigan State University	Dr. Larry Forney	East Lansing, MI
Michigan State University	Dr. Susan Masten	East Lansing, MI
Ministry Environment Czech Republic	Dr. Pavel Novak	Praha, CZECHOSLOVAKIA
Montana Department of Health and Environmental Sciences	Dr. Pat Newby	Helena, MT
Montana State University	Dr. Alfred Cunningham	Bozeman, MT
National Institute of Public Health & Environmental Protection	Dr. Esther Soczo	Bilthoven, THE NETHERLANDS
National Research Council, Biotechnology Research Institute	Dr. Rejean Samson	Montreal, QB
NETAC, University of Pittsburgh Applied Research Center	Dr. Val Kelmeckis	Pittsburgh, PA
New Jersey Institute of Technology	Dr. Gordon Lewandowski	Newark, NJ
New Mexico Health & Environment Dept.	Ms. Anna Richards	Santa Fe, NM
North Carolina State University	Dr. Robert Borden	Raleigh, NC
Norwegian Geotechnical Institute	Dr. Gijs Breedveld	Oslo, NORWAY
NSI Technology Services, R.S. Kerr Laboratory	Dr. William Ghiorse	Ada, OK
Oak Ridge Associated Universities	Dr. Howard Adler	Oak Ridge, TN
Oak Ridge National Laboratory	Dr. Steve Herbes	Oak Ridge, TN
OCRI	Mr. Gerry Turcotte	Kanata, ON
Orange County Health Department	Mr. James Strozier	Santa Ana, CA
Orange County Water District	Dr. Harry Ridgway	Fountain Valley, CA
Rice University	Dr. Chris Ward	Houston, TX
Rutgers University	Dr. Robert Ahlert	Piscataway, NJ
Rutgers University	Dr. Owen O'Connor	Piscataway, NJ
Rutgers University, Center for Agricultural Molecular Biology	Dr. Laura Meagher	New Brunswick, NJ
San Diego State University	Dr. Barbara Hemmingsen	San Diego, CA
Sandia National Laboratories	Ms. Sally Jo Raubfogel	Livermore, CA
South Dakota State University	Dr. Charles McMullen	Brookings, SD
Stanford University	Dr. Perry McCarty	Stanford, CA
Stanford University	Dr. D. Grbic-Galic	Stanford, CA
Stanford University	Dr. Lewis Semprini	Stanford, CA

Research Institution	Contact	Location
State of California, Water Branch	Dr. Manjulika Chakrabarti	Monterey Park, CA
State University of New York	Dr. Kenneth Hammel	Syracuse, NY
Tarleton State University	Dr. Ron Jones	Stephenville, TX
Technical University of Braunschweig	Dr. H.H. Hanert	Braunschweig, GERMANY
Technical University of Denmark, Dept. of Environmental Engineering	Dr. Erik Arvin	Lyngby, DENMARK
Technical University of Hamburg-Harburg	Dr. Rainer Stegman	Hamburg, GERMANY
Texas A&M University	Dr. Yavuz Corapcioglu	College Station, TX
The John Hopkins University	Dr. Edward Bouwer	Baltimore, MD
Tufts University	Dr. Wayne Chudyk	Bedford, MA
Tulane University	Dr. Sanjoy Bhattacharya	New Orleans, LA
U.S. Air Force	Mr. Johnny Combs	Dyass Air Force Base, TX
U.S. Air Force	Mr. Edward Hayes	Hill Air Force Base, UT
U.S. Army Toxic & Hazardous Materials Agency	Mr. Robert Bartell	Aberdeen Proving Ground, MD
U.S. Department of Energy	Mr. Tom Anderson	Washington, DC
U.S. EPA - Office of Research & Development	Mr. Steve Lingle	Washington, DC
U.S. EPA, Office of Research and Development (RD-681)	Mr. Michael Mastracci	Washington, DC
U.S. EPA, R.S Kerr Environmental Research Lab	Dr. Stephen Hutchins	Ada, OK
U.S. EPA, R.S Kerr Environmental Research Lab	Dr. John Wilson	Ada, OK
U.S.E.P.A., Office of Toxic Substances	Ms. Kay Austin	Washington, DC
U.S.E.P.A., Office of Toxic Substances	Mr. Ronald Evans	Washington, DC
U.S.E.P.A., Superfund Technology Demonstration Division	Dr. Ronald Lewis	Cincinnati, OH
UK Department of Trade & Industry	Dr. Roger Dunn	Stevenage, Hertfordshire, UK
University of Akron	Dr. Mark Kennedy	Akron, OH
University of Alberta	Dr. Phillip Fedorak	Edmonton, AB
University of Arizona	Dr. Bob Arnold	Tuscon, AZ
University of Arizona	Dr. Gary Amy	Tuscon, AZ
University of California, Riverside	Dr. D.D. Focht	Riverside, CA
University of California, Riverside	Dr. W.T. Frankenberger, Jr.	Riverside, CA
University of California, Santa Barbara	Dr. Deborah Kaska	Santa Barbara, CA
University of Groningen	Dr. Dick Janssen	Groningen, GERMANY
University of Helsinki	Dr. Max Haggblom	Helsinki, FINLAND
University of Idaho	Dr. Ronald Crawford	Moscow, ID

Appendix D. (Continued)

Research Institution	Contact	Location
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University of Iowa	Dr. D.T. Gibson	Iowa City, IA
University of Kansas	Dr. Clarence Buller	Lawrence, KS
University of Karlsruhe	Dr. Bruno Herrling	Karslruhe, GERMANY
University of Louisville	Dr. Ronald Atlas	Louisville, KY
University of Lowell	Dr. Clifford Bruell	Lowell, MA
University of Massachusetts	Dr. David Ostendorf	Amherst, MA
University of Massachusetts	Dr. M. Switzenbaum	Amherst, MA
University of Michigan	Dr. Tim Vogel	Ann Arbor, MI
University of Minnesota	Dr. H.C. Tsien	Navarre, MN
University of Missouri, Rolla	Dr. Purush TerKonda	Rolla, MO
University of New Hampshire	Dr. M.R. Collins	Durham, NH
University of New Mexico	Dr. Maribeth Watwood	Albuquerque, NM
University of North Carolina	Dr. F. Pfaender	Raleigh, NC
University of North Dakota	Dr. John Gallagher	Grand Forks, ND
University of Notre Dame, Center for Bioengineering and Pollution Control	Dr. James Earley	Notre Dame, IN
University of Oklahoma	Dr. Joseph Suflita	Norman, OK
University of Regina	Dr. Roy Cullimore	Regina, SK
University of Sheffield	Dr. A.J.M. Baker	Sheffield, S. Yorkshire, UK
University of Southern California	Dr. Joe Devinny	Los Angeles, CA
University of Tennessee	Dr. Tom Phelps	Knoxville, TN
University of Tennessee	Dr. David White	Knoxville, TN
University of Tennessee	Dr. G.S. Sayler	Knoxville, TN
University of Tennessee, Center for Environmental Biotechnology	Dr. Sue Markland Day	Knoxville, TN
University of Texas	Dr. Gerald Speitel, Jr.	Austin, TX
University of Tokyo	Dr. Naoki Ouchiyama	Tokyo, JAPAN
University of Tokyo	Dr. Ohgaki	Tokyo, JAPAN
University of Toronto	Dr. Brent Sleep	Toronto, ON
University of Washington	Dr. Barbara Denovan	Edmonds, WA
University of Waterloo	Dr. Colin Mayfield	Waterloo, ON
University of Waterloo	Dr. James Barker	Waterloo, ON
University of Wisconsin, Madison	Dr. Patrick Eagan	Madison, WI

Research Institution	Contact	Location
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Utah State University	Dr. Michael McFarland	Logan, UT
Vanderbilt University	Dr. Richard Speece	Nashville, TN
Ventura County Environmental Health Division	Mr. Douglas Beach	Ventura, CA
Wageningen Agricultural University	Dr. A.J.B. Zehnder	Wageningen, THE NETHERLANDS
Wageningen Agricultural University	Dr. Wil de Bruin	Wageningen, THE NETHERLANDS
Wastewater Technology Centre	Mr. Rob Booth, P.Eng.	Burlington, O
Dr. Carol Litchfield		Newark, DE